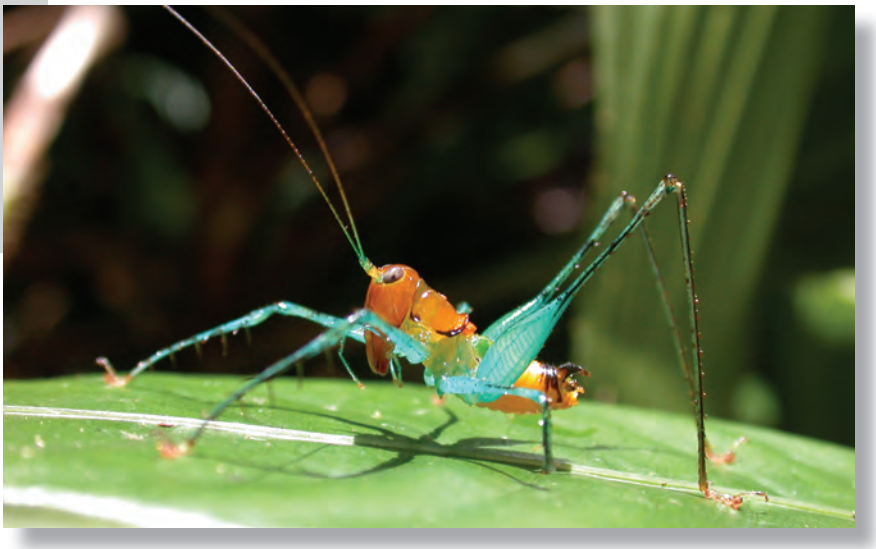
Abc Taxa

Manual on field recording techniques and protocols for All Taxa Biodiversity Inventories and Monitoring

Edited by:
J. Eymann, J. Degreef, Ch. Häuser,
J.C. Monje, Y. Samyn and D. VandenSpiegel



Volume 8, part 2 (2010)

Editors

Yves Samyn - Zoology (non African)

Belgian Focal Point to the Global Taxonomy Initiative
Royal Belgian Institute of Natural Sciences
Rue Vautier 29, B-1000 Brussels, Belgium
yves.samyn@naturalsciences.be

Didier VandenSpiegel - Zoology (African)

Department of African Zoology
Royal Museum for Central Africa
Chaussée de Louvain 13, B-3080 Tervuren, Belgium
dvdspiegel@africamuseum.be

Jérôme Degreef - Botany

Belgian Focal Point for the Global Strategy for Plant Conservation
National Botanic Garden of Belgium
Domaine de Bouchout, B-1860 Meise, Belgium
jerome.degreef@br.fgov.be

Instructions to authors

http://www.abctaxa.be

ISSN 1784-1283 (hard copy)
ISSN 1784-1291 (on-line pdf)
D/2010/0339/3

Manual on field recording techniques and protocols for All Taxa Biodiversity Inventories (ATBIs), part 2

Edited by

Jutta Eymann
Staatliches Museum für Naturkunde Stuttgart
Rosenstein 1, 70191 Stuttgart, Germany
Email: eymann.smns@naturkundemuseum-bw.de

Jérôme Degreef
National Botanic Garden of Belgium
Domaine de Bouchout, B-1860 Meise, Belgium
Email: jerome.degreef@br.fgov.be

Christoph L. Häuser
Museum für Naturkunde
Invalidenstr. 43, 10115 Berlin, Germany
Email: Christoph.Haeuser@mfn-berlin.de

Juan Carlos Monje
Staatliches Museum für Naturkunde Stuttgart
Rosenstein 1, 70191 Stuttgart, Germany
Email: monje.smns@naturkundemuseum-bw.de

Yves Samyn
Royal Belgian Institute of Natural Sciences
Vautierstraat 29, B-1000 Brussels, Belgium
Email: yves.samyn@naturalsciences.be

Didier VandenSpiegel
Royal Museum for Central Africa
Chaussée de Louvain 13, B-3080 Tervuren, Belgium
Email: dvdspiegel@africamuseum.be

Cover illustration: *Arachnoscelis* sp. [Orthoptera Tettigoniidae, predator] found in San Lorenzo forest, during the large-scale biotic inventory IBISCA-Panama (Photo by Maurice Leponce).

Table of contents, part 2

Chapter 13

Sampling of bryophytes

by

Alain Vanderpoorten
Research associate, Belgian Funds for Scientific Research
University of Liège, Institute of Botany B22, Sart Tilman, B-4000 Liège, Belgium
Email: A.Vanderpoorten@ulg.ac.be

Beáta Papp
Hungarian Natural History Museum, Botanical Department
Budapest, Pf. 222, H-1476, Hungary
Email: pappbea@bot.nhmus.hu

Robbert Gradstein
Albrecht von Haller Institute of Plant Sciences, Department of Systematic Botany
Untere Karspüle 2, D-37073 Göttingen, Germany
Email: sgradst@uni-goettingen.de

Abstract

In this chapter, we provide practical guidelines for collecting and recording bryophytes. Bryophyte species exhibit a high specificity to meso- and microhabitat conditions and, although some can be observed all year-round, many are annual and/or can be identified only during a short period of the year. Completely random plot sampling (RS) or systematic sampling (SS) are therefore likely to miss important types of variation within the sampling area unless the intensity of the sampling (*i.e*. number of plots and number of visits at different seasons) is very high. Therefore, it is appropriate to use a sampling methodology, such as Floristic Habitat Sampling (FHS), that focuses on mesohabitats as the sampling unit. SS and RS offer, however, substantial advantages over FHS in terms of statistical comparisons across plots. Therefore, the combination of a systematic grid, usually of 1 to a few km², within which FHS is performed, is recommended. The size of the sampling plot is discussed depending on the goals that are followed. For recording rare species, the Area of Occupancy (AOO), defined as the area calculated by summing up all 2 x 2 km grid squares actually occupied by a taxon, is used by IUCN as a standard measure for defining species frequency. In the case of bryophytes, however, it is strongly advisable to decrease the mesh size because AOO values decline sharply as the scale of measurement reduces, as a result of the linear and frequently fragmented distribution of the species. Scientific collecting is still essential for a number of reasons, including specimen identification and herbarium collections for taxonomic studies – which is especially true for bryophytes because, although the larger species can often be named in the field, many are distinguished based on microscopic characters – and, more recently, for the constitution of DNA libraries. The collecting techniques, including information on what and how much to collect in the field, how to pack, label, dry and process specimens, are finally reviewed.

Key words: bryophyte, moss, liverwort, hornwort, floristic habitat sampling, random sampling, plot sampling, phenology, diversity

1. Introduction

Bryophyte is a generic name for plants characterized by a life-cycle of alternating haploid and diploid generations with a dominant gametophyte. They include the liverworts, mosses, and hornworts. Liverworts and hornworts comprise about extant 5,000 and 300 species, respectively. Together with mosses, which, with approximately 12,000 species, are the second most diverse phylum of land plants, bryophytes thus include a substantial proportion of the total biodiversity of land plants.

Although bryophytes are rarely the most conspicuous elements in the landscape, they play important ecological roles in terms of water balance, erosion control, or nitrogen budget, or simply by providing habitat for other organisms. Furthermore, bryophytes locally exhibit richness levels that are comparable or even higher than those of angiosperms. Lastly, and perhaps most importantly, although global biodiversity patterns tend to be congruent across taxa, especially ß diversity patterns (Schulze *et al.*, 2004; Kessler *et al.*, 2009), diversity patterns in bryophytes do not necessarily follow the patterns present in other, better-studied taxa, so that an enlarged concept of biodiversity has become increasingly necessary. As a result, there has been an increasing awareness of the necessity to include cryptogams in general, and bryophytes in particular, in conservation programs and biodiversity assessments.

In this chapter, we attempt at providing practical guidelines for collecting and recording bryophytes. From recent specialized textbooks (Goffinet & Shaw, 2009; Vanderpoorten & Goffinet, 2009), we briefly summarize the biological and ecological features of bryophytes that are relevant to their study in the field. We then review, based upon information provided in many specialized field guides, to which we refer for further information (O'Shea, 1989; Gradstein *et al.*, 2001; Wigginton, 2004), the sampling strategies and collecting techniques that are most appropriate for recording bryophyte diversity.

2. Where and when to collect bryophytes?

2.1. Where do bryophytes occur?

Bryophytes are generally seen as small plants confined to humid habitats, avoiding exposure to direct sunlight. Yet, an alert naturalist will quickly notice their presence in virtually every ecosystem. In parts of the world where short growing seasons limit plant growth, bryophytes, and especially mosses, may dominate the vegetation. Similarly, in temperate and tropical rain forests, bryophytes, and especially liverworts, compose luxuriant epiphytic communities that play important ecological functions, especially in terms of water and nutrient flow. Even in modern cities where air pollution and the man-made environment may seem unrelenting, bryophytes are able to colonize crevices in masonry.

The diversity of bryophytes is correlated with habitat heterogeneity at two spatial scales. Mesohabitats are localized physiographic (*e.g.* streams, seeps, cliffs) or physiognomic (*e.g.* forests) features. In a forested landscape, mesohabitats are

arranged into a mosaic of dominant mesohabitats (*e.g.* forests), wherein restricted mesohabitats (*e.g.* streams, seeps, cliffs) exist (Vitt & Belland, 1997). Microhabitats (*e.g.* trees, logs, rocks, stumps) are the smallest landscape units and may be unique to one type of mesohabitat. Epiphytic communities provide a classical example of microhabitat differentiation. Epiphytes typically exhibit both a vertical and a horizontal zonation, segregating vertically from the base to the crown along gradients of humidity, pH, and nutrient content (Barkman, 1958; Sillett & Antoine, 2004). Within each ecological unit, bark microtopography further generates a mosaic of microhabitats. For example, Barkman (1958) described the mosaic of species inhabiting beech bark in The Netherlands (Fig. 1). Wound exudates induce a vertical zonation of neutrophytic species, including *Orthotrichum diaphanum, Syntrichia laevipila* and *Zygodon viridissimus*, which are normally absent from acid beech bark. The last two species grow lower, presumably due to greater moisture near the ground. In contrast, acidophilous species, such as *Lophocolea heterophylla*, develop far from the wound.

Different species thus tend to utilize different portions of the resource continuum available. The competitive exclusion principle predicts that species avoid competition by occupying different niches, creating a spatial pattern that represents habitat partitioning corresponding to habitat heterogeneity. Thus, an increasing body of literature points to the strong correlation between habitat and species diversity. Some habitats are, however, more species-rich than other and hence, request a longer investigation time. Bryophytes are poikilohydric, which means that they suspend any metabolic activity upon drying. They tend therefore to be more dominant in sheltered, humid habitats than on open ground directly exposed to irradiation and desiccation.

A good trick to find species-rich habitats is to look at the extent of species cover. There is indeed a positive correlation between carpet density and species diversity for two main reasons. First, massive cover suggests that the habitat has the appropriate humidity level for many species to establish. Second, at low to moderate densities, growth is constrained by water availability. Moderately dense stands are dehydrated less rapidly than loose stands or isolated shoots because a dense packing of shoots may reduce water loss by effectively reducing the diameter of capillary spaces among close neighbours. Bryophytes growing in dense communities are therefore able to remain physiologically active for a larger part of the growing season, resulting in greater biomass and diversity.

2.2. Can we record bryophytes all year-round?

It is often believed that bryophytes occur all year-round, and this is one of the reasons why many naturalists shift to bryology in wintertime. This is definitely true for stress-tolerant species, which invest much in gametophytic development, enabling them to survive periods of stress. As a most extreme example, large cushions of the moss *Leucobryum glaucum* on forest ground or *Sphagnum* species in peat bogs, all of which occur in stable habitats and display gametophytic adaptations to store water in dead hyaline cells, can last for centuries. Thus, bryophyte species of long-lived, stable mesohabitats such as woodlands, can in fact be recorded at any time.

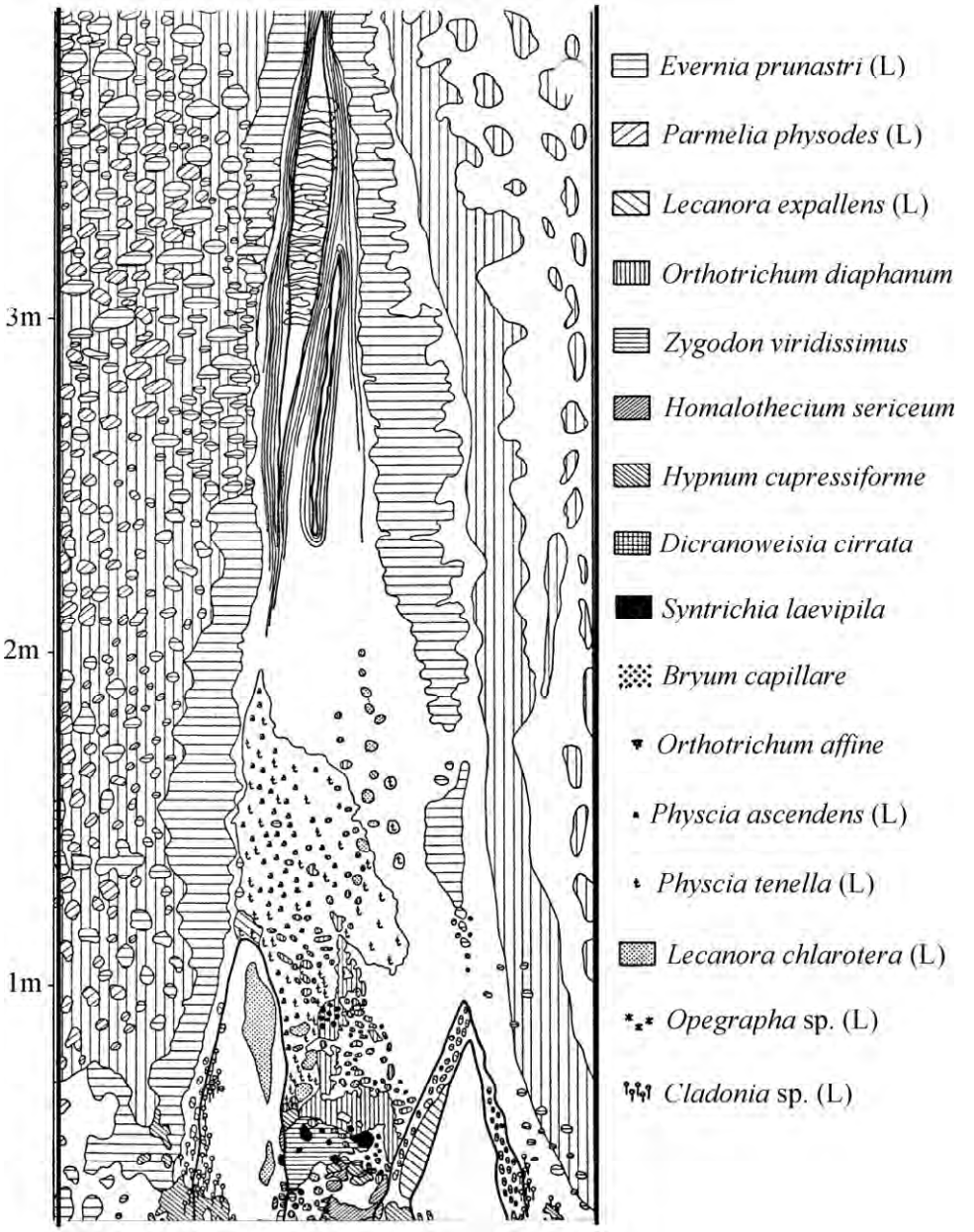


Fig. 1. Mosaic of cryptogamic vegetation comprised of lichens (L) and bryophytes along the first 4 m on an old beech trunk in The Netherlands (after Barkman, 1958).

It must be emphasized, however, that whilst perennial species can be observed regardless of the season, their identification might rely on sporophytic features that can be observed only during a short period of the year. The moss genus *Orthotrichum*, for example, includes mostly perennial epiphytic species whose identification relies on specific sporophyte features. In the northern hemisphere, the capsule reaches its full development in the spring, and taxonomically relevant characters of the peristome progressively become impossible to observe towards the summer season, during which the capsule itself eventually falls down.

In many other habitats, bryophyte species can be observed during a short period of the year only. In fact, plants have to cope with unstable habitats in time (*e.g.,* seasonal climate variations) and space (*e.g.*, habitat degradation or destruction). To face the risk of local extinction, they may either disperse in an attempt to establish new populations or remain under the form of long-lived diaspores, from which new establishment will be subsequently possible under favourable growth conditions. Parts of these diaspores may become buried into the soil, requiring light for germination, constituting a bank of diaspores. Because of the vulnerability of their gametophyte, bryophytes are, in particular, likely to rely more on stored propagules for their long-term survival than seed plants. Species of unstable habitats that recur predictably at a given site thus tend to produce a few, large spores with a low dispersal capacity but better chances of successful establishment and a longer life span in the diaspore bank. This is, for example, the case of hornworts in temperate areas, which are well adapted to regular disturbance in arable fields thanks to their diaspore bank, or of annual thalloid liverwort communities in xerotropical environments experiencing a severe drought season. On a less regular basis, habitats such as dried-out ponds are quickly recolonized thanks to the diaspore bank and their survey is often rewarded by the discovery of many specialized species.

As a result, all habitats cannot be recorded all year-round and some must be investigated during the appropriate season. During a survey of the bryophytes of arable land in Britain and Ireland for example, inventorying of the fields occurred at a time of year when the bryophytes were large enough for most of them to be identified or, in the rare cases of fields with no bryophytes, at a time of year when bryophytes would have been identifiable if present. In practice, this meant that fields were inventoried in the autumn, winter and early spring (Preston *et al.*, in press).

3. How to record bryophytes?

3.1. How to organize the sample plots?

An appropriate sampling methodology is crucial to understanding patterns of community and taxon diversity at the landscape scale. The type of sampling used for estimating diversity depends on the organism being studied, how closely that organism is associated with its substrate, and the nature of the ecological question (Krebs, 1989). In plant studies, Clements (1905) described methods for collecting plant species data using plots. Since that time, many variations of quantitative measurements using plots have been used. The bounded nature of

plots in relation to a specific sample area allows for quantitative sampling of species abundance and frequency, and later statistical analysis. This has made plot sampling a successful method for studying population and community dynamics in bryophytes and many other groups of plants.

Plots may be organized in a regular fashion, using a systematic grid, or selected at random. For instance, the combination of a systematic grid of 10 x 10 km, within which 'standard relevés' of 100 m^2 are inventoried, has been used for the standardized mapping of Swiss bryophytes (Urmi *et al.*, 1990). In each 'relevé', all bryophyte species are collected and determined, and voucher specimens are kept. This approach is most appropriate to identify the commonest species and assess their frequency and distribution, but may not allow for the recording of rare species. This is because many bryophyte species exhibit a high specificity to peculiar meso- and microhabitat conditions; a completely random plot sampling method is likely to miss important types of variation within the sampling area unless the intensity of the sampling (*i.e.* number of plots) is very high. Therefore, it is appropriate to use a sampling methodology that focuses on mesohabitats as the sampling unit. Sampling methods aimed at assessing total bryophyte diversity studies should include all of the potential habitats in an ecosystem. The method referred to as Floristic Habitat Sampling (hereafter, FHS) uses mesohabitats as the basic sampling units.

Comparisons of the efficiency of random Plot Sampling (hereafter, PS) and FHS suggested that the latter captures a greater mean species richness per stands than PS (Newmaster *et al.*, 2005). Bryophyte diversity estimates compared within the dominant forest mesohabitat were found to be much greater (*i.e.* species richness is 50% higher) when using FHS as compared to PS (Fig. 2). Although it is not made explicit, and although other data from herbarium records as well as casual observations are also included, FHS within each square of a systematic grid of one to several km is basically used in most of the European mapping programs for example in the UK (Hill *et al.,* 1991-1994), The Netherlands (van Tooren & Sparrius, 2007), Germany (Meinunger & Schröder, 2007), and Belgium (Sotiaux *et al.*, 2000; Sotiaux & Vanderpoorten, 2001, 2004).

Usually, all mesohabitats are identified from the analysis of fine-scale topographic maps. Each mesohabitat is then visited and sampled until no new species are reported. In some instance, special attention is paid to key-habitats that are identified on the basis of specific attributes, *e.g.* the known presence of rare bryophytes, special topography or soils, or, since the diversity of bryophytes most often correlates with global biodiversity patterns (Pharo *et al.*, 2000; Schulze *et al.*, 2004), the known presence of rare taxa.

The time necessary to survey an area depends of course of many factors including the number and experience of recorders, as well as the extrinsic floristic quality of the habitats. In Belgium, our experience is that the record of a grid-square of 4 x 4 km is considered complete, *i.e.* with no more than approximately 10% of missed species, takes between one (species-poor squares with low habitat heterogeneity, with approximately 50-60 species/square) and four days (species-rich squares with high habitat heterogeneity and quality with >150 species/square).

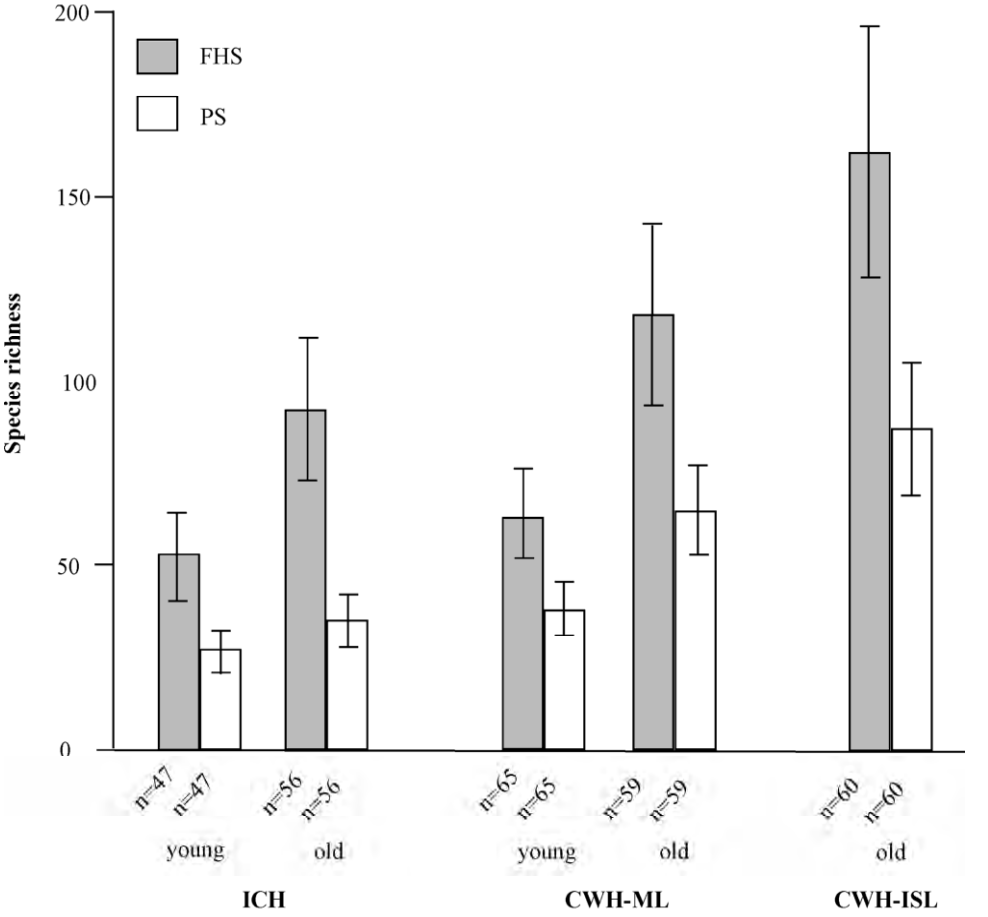


Fig. 2. Alpha diversity of stands assessed using floristic habitat sampling (FHS including all mesohabitats) and plot sampling (PS). Cedar hemlock forests are divided into inland (ICH), coastal mainland (CWH-ML), coastal oceanic (CWH-ISL), and by age classes (class 4, young = 80 years and class 9, old > 250 years). Error bars represent two standard errors on either side of the mean (reproduced from Newmaster *et al.,* 2005 with permission from Blackwell).

3.2. What size should sample plots have?

The size of the sampling plot depends on the goals that are followed. For biodiversity inventories, large plots should be favored since species richness typically increases with sample area (Fig. 3). In a comparative study of bryophyte forest diversity in Canadian forests, Newmaster *et al.* (2005) found that the 20 m-diameter plot used in the PS method sampled 314 m^2 of forest mesohabitat resulting in a mean species richness of 35 (± 5) species. Expanding sampling area to 1000 m^2 increased mean species richness by only 18 species.

Furthermore, species richness steadily increases even after 5000 m^2 has been sampled, increasing mean species richness in the dominant forest mesohabitat to just over 80 (± 6) species (Fig. 3). Using FHS, the mean species richness within the dominant forest mesohabitat was 106 (± 9) species. In fact, intensifying PS or simply sampling large areas using randomly placed plots will not necessarily include the natural variety in microhabitats. This is because PS within a mesohabitat will exclude important microhabitats and their respective bryophyte communities even after sampling unconventionally large sample areas. These results clearly suggest that the size of the sampling units depends on the sampling strategy itself, and that, in any case, the size of each sampling unit should be determined by means of species-area curves. In tropical rain forest, Gradstein *et al.* (2003) found that full sampling of 4-5 mature trees may yield 75-80% of the tree-inhabiting bryophytes in a forest stand (excluding epiphylls).

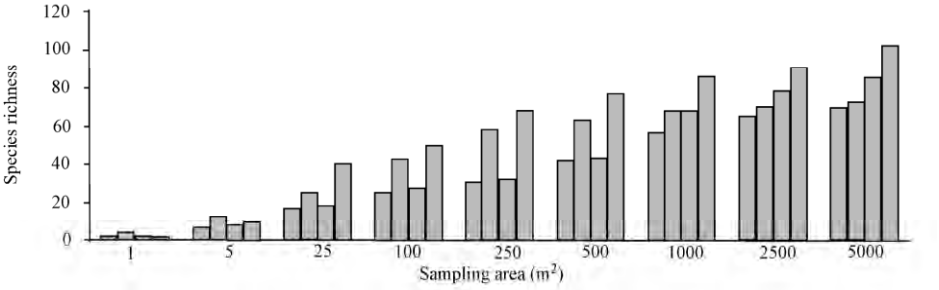


Fig. 3. Mesohabitat alpha diversity (species richness) within increasing sample size areas for 287 temperate rainforest stands (SP = seep, CF = cliff, FS = forest, ST = stream) (reproduced from Newmaster *et al.,* 2005 with permission from Blackwell).

For the record of rare species, the Area of Occupancy (AOO), which is defined as the area, calculated by summing up all grid squares with the mesh size of 2 x 2 km that are actually occupied by a taxon, excluding cases of vagrancy, is used by IUCN as a standard measure for defining species frequency. In the case of bryophytes, however, it is strongly advisable to decrease the mesh size because AOO values decline sharply as the scale of measurement reduces, as a result of the linear and frequently fragmented distribution of the species (Callaghan, 2008).

3.3. What to measure in each plot?

Depending on the time available and the goals followed, presence-absence or increasingly complex abundance indices can be used to document the frequency of each species in each sampling unit. The 'relevé' sampling method involves the attribution, to each species within the plot, of a coefficient of abundance-dominance, sometimes associated with a coefficient of sociability (see chapter on vascular plant recording), which serve to describe the cover of each species on the ground and its distribution mode, from lose, isolated plants to densely packed cushions.

In some tropical areas characterized by a very lush and species-rich bryophyte vegetation, however, this method may not be applicable and alternative

strategies must be used. One such strategy is to sub-divide each sampling unit into smaller sub-plots of a few dm², select some at random, perform complete species lists in each, and assess the frequency of each species across the sub-plots in each sampling unit. Alternatively, the same procedure of sub-division of the main sampling unit can follow a systematic scheme. This is, for instance, the method applied by the Hungarian Bryophyte Monitoring Program (Papp *et al.*, 2005) for the record of epiphytes. Within each sampling unit, each standing tree (living or dead) with a diameter of at least 19 cm at breast height is included in the sampling of epiphytic bryophyte vegetation. The sampling of epiphytic bryophytes is carried out at three levels: 10 cm (1. level), 70 cm (2. level), 140 cm (3. level) upwards from the base of the tree. A 10 cm wide cylinder is examined at each level (from the marked level 5-5 cm upward and downward), where the occurrences of the species are recorded (presence/absence data).

A protocol for rapid and representative sampling of epiphytic bryophytes growing on bark of trees in tropical rain forest was designed by Gradstein *et al.* (2003). Within a core area of one hectare, 5 mature rain forest trees (standing well apart and differing in bark structure) are sampled from the base to the outer canopy using the single rope technique (ter Steege & Cornelissen, 1988) or some other method for sampling of the forest canopy. Species are collected in 4 small plots within each of 6 height zones, the so-called “Johannson zones” (1: tree base, 2a: lower trunk, 2b: upper trunk, 3: lower crown, 4: middle crown, 5: outer crown). Plots in zones 1-3 are 20 x 30 cm and positioned in each cardinal direction, those on thin branches in zones 5-6 are ca. 60 x 10 cm long and positioned on the upper and lower surfaces of the branch. For safety reason, plots in zones 4 and 5 are sampled on the ground from cut-off branches.

A protocol for sampling of epiphyllous bryophytes in tropical rain forest was designed by Lücking & Lücking (1996).

4. Collecting techniques

Scientific collecting is essential for a number of reasons, including specimen identification, herbarium collections for taxonomic studies, and, more recently, constitution of banks of DNA. This is especially true for bryophytes because, although the larger species can often be named in the field with a 10-20x hand-lens, many are distinguished based on microscopic characters. Reference collections of specimens are thus invaluable in the study of bryology, but in order to obtain useful specimens for research, the correct techniques for collecting and processing should be employed. It must also be emphasized that, although bryophyte species rarely legally protected, it is necessary to obtain permits to collect bryophytes and an export licence if the material is to be taken out of the country. Herbarium staff can often advise on what is needed, but obtaining necessary papers and permissions can be a lengthy process, so should be investigated well in advance.

4.1. Packeting

Bryophytes are among the easiest plants to collect (Buck & Thiers, 1996). Since they lack roots, they can often be readily collected by hand, although some species closely attached to their substrate will have to be scratched using a knife. Specimens should be selected to include all the parts of the plant needed for identification. Sporophytes are often useful, if not necessary, for identification, and should be searched for. Several mosses from unstable habitats, *e.g.* riverbanks, arable fields, have rhizoidal tubers buried in the soil. As these are often diagnostic, these bryophytes should be collected with 1-3 cm of the substrate (Whitehouse, 1966; Porley, 2008).

Individual species within a collection should be packed-up separately, so far as this is possible. It is in fact generally easier when the material is still fresh than later, when several collections jumbled together in a single packet have to be separated. The specimens are normally put into envelopes. A standard envelope can be folded from an A4 paper to be (10-)12 x 14 cm in size (Fig. 4). Particularly small specimens should be wrapped separately in mini-packets before being put into normal size packets. If sporophytes or fertile structures are rare, these should also be placed in mini-packets, but attached to a piece of the gametophyte to avoid any subsequent confusion. If specimens are very wet, as is often the case with *Sphagnum*, they should be gently pressed to remove most of the water, and packed into a double or treble thickness packets. As for ground-dwelling species, it is often more appropriate to keep them in stiff boxes for transportation and storage to avoid ending up with a mixture of soil particles and plant fragments.

For collecting of epiphyllous bryophytes in tropical rain forest, whole leaves on which the epiphylls are growing are collected in new papers in a plant press, lightly pressed and dried. The epiphyllous species are subsequently sorted, and leaves cut up, in the laboratory using a dissecting microscope. For collecting of thalloid liverworts and hornworts it may also be recommendable to dry the specimens in a plant press instead of in collecting bags, in order to keep them flat and avoid them from becoming rolled inwards. Pressing of the specimens should be lightly only, to avoid damage to the plants.

4.2. How much to collect?

Collecting of specimens for scientific purposes is usually highly selective and seldom constitutes a real threat to the survival of species. The extinction of species by a targeted over-collecting has been, however, already documented. It is difficult to provide exact guidelines since everything depends on species size, local and overall abundance, etc. As a general rule, collecting enough to fill a 12 x 8 cm packet should be plenty for a robust species. On the other hand, too small specimens are of no value if there is insufficient material to allow identification and, perhaps, DNA extraction. In addition, the really important plant in a collection may not be what the collector actually saw in the field, but some minute plant sparsely mixed with it, and only discovered later in the laboratory.

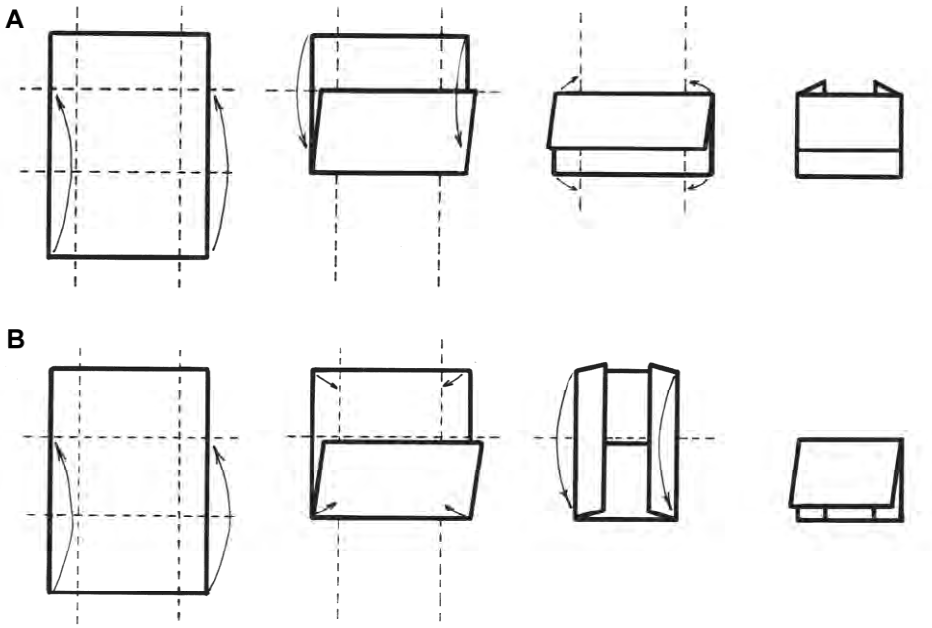


Fig. 4. Folding procedure for packing-up bryophytes.

4.3. Data and labelling

The information record is similar to that of other plants, and includes habitat information (for instance, if a species occurs on tree or rock, the tree species or rock type should be recorded), nature of the surrounding vegetation, elevation, and locality details, including GPS coordinates. For rare species, information on population size is often useful but might be difficult to assess in the case of bryophytes. Indeed, many bryophyte species are highly clonal, and several gametophytes can develop from a single protonema following the germination of a single spore.

Thus, what is the entity that best corresponds to discrete individuals like animals? For practical reasons, a purely pragmatic definition can often be used. For species that depend on discrete substrate entities (such as tree trunks or droppings), each substrate entity can be considered to contain one or two individuals. For bryophyte species growing on ground or rocks, one individual may be assumed to occupy a surface of 1 m^2. However, in some rare cases of some very small mosses (*e.g.* the genera *Seligeria* and *Tetrodontium*), one individual might be associated with a surface of 0.1 m^2.

4.4. Drying and processing

The collected specimens should be dried as soon as possible to avoid fungal damage. In most cases, the packets can be left to air-dry. In wet areas during extended expeditions, however, drying might become a major issue and

preoccupation, and the use of a plant dryer can sometimes become necessary (Frahm & Gradstein, 1986). As liverwort capsules tend open when drying, releasing their spores, it is recommended that some specimens with capsules be placed in a small paper envelope before drying together with the rest of the sample, to ensure that at least some unopened capsules are preserved.

These is no need to give a descriptive account of the plant, as one does systematically for fungi and sometimes for higher plants, since most bryophyte species recover their primary appearance upon remoistening. A special care must, however, be taken with liverworts. Indeed, the identification of many species relies on the size, shape, number, colour, and distribution of oil bodies, which are unique organelles among land plants. Because of the volatility of the oils they contain, oil bodies progressively disappear upon drying in the laboratory. In some taxa, the process takes only a few hours, so that fresh material must be studied, whereas in other, oil-bodies last for some years and can still be studied on herbarium specimens. In any case, it is advisable to take a micro-photograph of the cells to keep a record of the oil body morphology.

For preservation of DNA, fresh material should be cleaned and quickly air-dried, and subsequently kept dry. Any moistening of the material must be avoided as this might lead to degradation of the DNA, making the material unsuitable for molecular analysis.

5. Acknowledgements

The authors sincerely thank Herman Stieperaere and an anonymous reviewer for constructive comments on the manuscript.

6. References

BARKMAN, J.J. 1958. *Phytosociology and ecology of cryptogamic epiphytes*. Van Gorcum, Assen: 628 pp.

BUCK, W.R. & THIERS, B.M. 1996. Guidelines for collecting bryophytes. *In*: ALEXIADES, M.N. (Ed.). *Selected Guidelines for Ethnobotanical Research: a Field Manual*. New York Botanical Garden, New York: 143-146.

CALLAGHAN, D.A. 2008. Scale dependency and area of occupancy: *Tortula freibergii* in north-west England. *Journal of Bryology* 30: 279-282.

CLEMENTS, F.E. 1905. *Research methods in ecology*. Woodruff-Collins, Lincoln: 334 pp.

FRAHM, J.P. & GRADSTEIN, R. 1986. An apparatus for drying bryophytes in the field. *Bryological Times* 38: 5.

GOFFINET, B. & SHAW, A.J. 2009. *Bryophyte Biology*, 2nd ed. Cambridge University Press, Cambridge: 565 pp.

GRADSTEIN, S.R., NADKARNI, N.M., KRÖMER, T., HOLZ, I. & NÖSKE, N. 2003. A protocol for rapid and representative sampling of epiphyte diversity of tropical rain forests. *Selbyana* 24: 87-93.

GRADSTEIN, S.R., CHURCHILL, S.P. & SALAZAR ALLEN, N. 2001. *Guide to the Bryophytes of Tropical America*. New York Botanical Garden, New York: 577 pp.

HILL, M.O., PRESTON, C.D. & SMITH, A.J.E. 1991-1994. *Atlas of the Bryophytes of Britain and Ireland*. Harley Books, Colchester: 1170 pp.

KESSLER, M., ABRAHAMCZYK, S., BOS, M., BUCHORI, D., PUTRA, D.D., GRADSTEIN, S.R., HÖHN, P., KLUGE, J., OREND, F., PITOPANG, R., SHAHABUDDIN, S., SCHULZE, C.H., SPORN, S.G., STEFFAN-DEWENTER, I., TJITROSOEDIRDJO, S.S. & TSCHARNTKE, T. 2009. Alpha and beta diversity of plants and animals along a tropical land-use gradient. *Ecological Applications* 19: 2142-2156.

KREBS, C.J. 1989. *Ecology*. Harper & Row, Dawn Mills, ON: 688 pp.

LÜCKING, A. & LÜCKING, A. 1996. Foliicolous bryophytes and lichens. *Ecotropica* 2: 67-71.

MEINUNGER, L. & SCHRÖDER, W. 2007. *Verbreitungsatlas der Moose Deutschlands*. Regensburgische Botanische Gesellschaft, Regensburg: Band 1: 636 pp.; Band 2: 699 pp.; Band 3: 709 pp.

NEWMASTER, S.G., BELLAND, R.J., ARSENAULT, A., VITT, D.H. & STEPHENS, T.R. 2005. The ones we left behind: Comparing plot sampling and floristic habitat sampling for estimating bryophyte diversity. *Diversity and Distributions* 11: 57-72.

O'SHEA, B. 1989. *A guide to collecting bryophytes in the tropics*. British Bryological Society, Cardiff: 28 pp.

PAPP, B., ÓDOR, P. & SZURDOKI, E. 2005. Methodological overview and a case study of the Hungarian Bryophyte Monitoring Program. *Boletín de la Sociedad Española de Briología* 26-27: 23-32.

PHARO, E.J., BEATTIE, A.J. & BINNS, D. 2000. Vascular plant diversity as a surrogate for bryophyte and lichen diversity. *Conservation Biology* 13: 282-292.

PORLEY, R. 2008. *Arable bryophytes, a field guide to the mosses, liverworts and hornworts of cultivated land in Britain and Ireland*. Wild Guides, Old Basing: 140 pp.

PRESTON, C.D., HILL, M.O., PORLEY, R.D. & BOSANQUET, S.D.S. in press. Survey of the bryophytes of arable land in Britain and Ireland. I. A classification of arable field assemblages. *Journal of Bryology*.

SCHULZE, C.H., WALTERT, M., KESSLER, P.J.A., PITOPANG, R., SHAHABUDDIN, S., VEDDELER, D., MUHLENBERG, M., GRADSTEIN, S.R., LEUSCHNER, C., STEFFAN-DEWENTER, I. & TSCHARNTKE, T. 2004. Biodiversity indicator groups of tropical land-use systems: Comparing plants, birds, and insects. *Ecological Applications* 14: 1321-1333.

SILLETT, S.C. & ANTOINE, M.E. 2004. Lichens and bryophytes in forest canopies. *In*: LOWMAN, M.D. & RINKER, H.B. (Eds). *Forest canopies*, 2nd ed. Elsevier Academic Press, New York: 151-174.

SOTIAUX, A. & VANDERPOORTEN, A. 2001. Atlas bryologique du Brabant wallon. *Lejeunia* 167: 1-77.

SOTIAUX, A. & VANDERPOORTEN, A. 2004. Catalogue, atlas commenté et mesures de conservation des bryophytes du bassin hydrographique de la Semois (Belgique, France). *Lejeunia* 175: 1-49

SOTIAUX, A., SOTIAUX, O., VANDERPOORTEN, A. & DURWAEL, L. 2000. The distribution of bryophytes in the forest of Soignes (South of Brussels, Belgium). *Scripta Botanica Belgica* 18: 83 pp.

TER STEEGE, H. & CORNELISSEN, H. 1988. Collecting and studying bryophytes in the canopy of standing rain forest trees. *In*: GLIME J.M. (Ed.). *Methods in Bryology*. Hattori Botanical Laboratory, Nichinan: 285-290.

URMI, E., SCHNYDER, N., & GEISSLER, P. 1990. A new method in floristic mapping as applied to an inventory of Swiss bryophytes. *In*: BOHN, U. & NEUHÄUSEL, R. (Eds). *Vegetation and flora of temperate zones.* SPB Academic Publishing, The Hague, The Netherlands: 21-32.

VANDERPOORTEN, A. & GOFFINET, B. 2009. *Introduction to Bryophytes*. Cambridge University Press, Cambridge: 312 pp.

VAN TOOREN, B.F. & SPARRIUS, L.B. (Eds). 2007. *Voorlopige verspreidingsatlas van de Nederlandse mossen*. Bryologische en Lichenologische Werkgroep: 352 pp.

VITT, D.H. & BELLAND, R.J. 1997. Attributes of rarity among Alberta Mosses: Patterns and prediction of species diversity. *The Bryologist* 100: 1-12.

WHITEHOUSE H.L.K., 1966. The occurence of tubers in European mosses. *Transactions of the British Bryological Society* 5: 103-116.

WIGGINTON, M.J. (Ed.). 2004. E.W Jones's Liverwort and Hornwort Flora of West Africa. *Scripta Botanica Belgica* 30: 443 pp.

Chapter 14

Manual on Vascular plant recording techniques in the field and protocols for ATBI+M sites – Inventory and Sampling of specimens

by

Elke Zippel
Botanic Garden and Botanical Museum Berlin-Dahlem, Freie Universität Berlin
Königin-Luise-Straße 6-8, 14195 Berlin, Germany
Email: e.zippel@bgbm.org

Thomas Wilhalm
Naturmuseum Südtirol
Bindergasse 1, 39100 Bozen, Italy
Email: Thomas.Wilhalm@naturmuseum.it

Conny Thiel-Egenter
FORNAT
Universitätstrasse 47, 8006 Zürich, Switzerland
Email: conny.thiel-egenter@fornat.ch

Abstract

The methods applied by botanists and ecologists to record and describe the constantly changing diversity on earth are as varied as the vegetation and flora itself. Alongside this the literature covering these methods are numerous and diverse. The method used in the field is selected on the basis of the study aims, previous knowledge of geological, ecological and floristic features of the study area as well as the extent of the fieldwork.
This manual is an overview of methods and a basic introduction, aimed especially at beginners, to higher plant recording of any study area. It contains basic aspects of planning, carrying out and documenting an inventory project but focuses on practical work in the field, designing sample plots and preparation of herbarium specimens. Theoretical foundations, statistical approaches and analyses are not covered in this manual. Reference to further reading is not complete due to the extensive literature covering inventory methods.

Key words: Vascular plants, flora mapping, field work, methods

1. Introduction

Flora and vegetation (the species composition and the total plant community at a defined site) of vascular plants (ferns and spermatophytes) are the most easily recognizable results of abiotic, biotic and human impacts on the earth's surface. Vegetation on earth has an outstanding importance especially in terrestrial habitats. Plants are important primary producers, providing the basis for the food web, and habitat for numerous – sometimes highly specialized – animal and fungal communities. Due to the high value of vegetation as a bio-indicator, it is possible to use vegetation type to predict the occurrence of other organisms or abiotic conditions. These characteristics make the accurate inventory of the flora and vegetation of an area worthwhile for a broad range of issues in basic ecological and bio-geographical research. Flora and vegetation mapping has been used in the framework of scientific investigation of taxa, habitats and ecosystems as well as in the applied sciences for nature conservation and monitoring programs for round about hundred years.

In view of both the enormous diversity of flora and vegetation and the vast number of approaches and study objectives in this field of research there are innumerable methods and field study designs for, *e.g.,* selecting sampling sites, plot shape and size, recording species, as well as gathering species frequency and distribution data. Because of this it is difficult or often impossible to summarise data gathered from the literature and to compare them directly. To overcome this issue botanists should strive to improve fieldwork standards.

This chapter focuses on the fieldwork needed to carry out inventories and monitoring of vascular plant taxa. To inventory means recording every single taxon regardless of whether the taxon name is known to the fieldworker or not. For this purpose we need a specialised approach, different from those documented in the bulk of literature dealing with vegetation mapping which focus on methods to inventory dominant or frequent species or life-forms (*e.g.*, Braun-Blanquet, 1964; Ellenberg *et al.*, 1968; Müller-Dombois & Ellenberg, 1974; Daubenmire, 1968; Barbour *et al.,* 1999; Bonham, 1989; Elzinga *et al.*, 1998).

The first floristic maps, with just 13 grid squares, were produced in the Netherlands at the beginning of the last century (Goethart & Jongmans, 1902). Ostenfeld (1931) presented a combination of point and area mapping in "Danmarks Topografisk-Botaniske Undersögelse". In the last fifty years, many mapping projects have been initiated, *e.g.,* the "Atlas of the British Flora" (Perring & Walters, 1962), the "Mapping of Central Europe", which uses grid squares of 10' longitude and 6' latitude (about 12 x 10 km), (Niklfeld, 1972), or the "Atlas Florae Europaeae" on the base of 50 x 50 km grids. Over the decades, an increasing number of publications have focused on methods and standards of flora and vascular plant diversity mapping (*e.g*., Niklfeld, 1978; Magurran, 1988; Wilson, 1988; Soulé & Kohm, 1989; Økland, 1990; Peters & Lovejoy, 1992; Stohlgren, 1994; Peterson *et al.*, 1995; Dallmeier & Comiskey, 1996; Nusser & Goebel, 1997; Ashton, 1998; Krebs, 1999; Hill *et al.*, 2005; Rich *et al.*, 2005).

Widely accepted standards for fieldwork techniques for species inventory do not exist. Only a few studies have investigated the accuracy, efficiency, and validity

of different methods (see overview in Stohlgren, 2006). The detailed study to consider standards for mapping and other conservation methods was published in Germany (Plachter *et al.*, 2002). An outstanding example of a detailed manual is given by Bergmeier (1992), which is based on 20 years of experience from the Central European floristic mapping project.

Monitoring of flora and vegetation, usually based on mapping projects, is becoming more and more important, particularly in the context of increasing extinction worldwide and accelerating climate change (*e.g.*, Campbell *et al.,* 2002; Pereia & Cooper, 2006; Cleland *et al.*, 2007; Kull *et al.*, 2008). Monitoring the biodiversity of an area involves regularly recording data at a site using defined recording methods. Monitoring studies may be applied at the level of landscape, ecosystem, species, population or genetic diversity (Noss, 1999) and provides data to observe long-term changes in plant diversity. A detailed manual for monitoring standards of endangered vascular plant species in the UK with many descriptive case studies is provided by JNCC (2004), a general overview about planning, methods and realisation in Hill *et al.* (2005).

This manual aims to convey the general principles and basic methods of flora mapping and monitoring. It is written for students and other beginners in the field with basic taxonomical and ecological knowledge. We focus on the inventory and monitoring of biodiversity expressed by the composition of vascular plants species visible above ground at the time of fieldwork in a given area and recorded metrics may include species abundance, frequency, and cover. For practical reasons, the soil seed bank is not taken into consideration. Likewise, neither the genetic diversity nor the diversity of plant communities are covered in this manual.

Completing an inventory of vascular plant flora for a region includes several key activities in the field: recording taxa and related data and making herbarium specimens. The taxon list should be accompanied by herbarium specimens, as well as geographical and accurately observed ecological data from the site and metadata (collector's name, institution, expedition, ...).

2. Inventory of vascular plant taxa

2.1. General comments

When beginning fieldwork planning one should bear in mind the why this work is proposed. The following questions of particular importance should be addressed: How large is the study area? Which infraspecific taxonomic levels ought to be considered, *i.e.,* should subspecies, varieties, and microspecies be recorded? How much time and what personnel resources are available? What monitoring intervals are needed?

The sampling strategy depends on the questions posed above. In fact, one must consider if it is feasible to explore the whole area or whether representative sample plots within the investigation area or transects along ecological gradients are necessary to sufficiently survey the flora. How many sample plots are needed and where should they be located? What is the best plot size and shape? What

additional environmental data should be recorded and what methods are to be applied for this purpose? Are there locals who know the area and are willing to provide support?

Several factors increase the likelihood of a complete inventory. These include smaller and more homogeneous investigation areas or sample plots, the experience of the observer, the amount of sampling and the time invested.

Collection permit

All fieldwork, visits to conservation areas, and collections must be made legally. If you work in protected areas or need to collect endangered or protected plants do not forget to ask the responsible authorities for the collecting permission.

2.2. Investigation season

In most cases it is not feasible to completely inventory all plant species in a single excursion. In fact, for a full inventory of the vascular plant flora it is crucial to consider the different phenological aspects of the flora during the growing season. For instance, geophytes are often underrepresented in mapping projects because they appear mainly either before or after the main growing season. Therefore, selecting the time of fieldwork is an important issue. If only one visit to the study area is possible, it is obvious that this should take place at the peak of the growing season when most species are in flower ('peak phenology') so as to observe as many species as possible and to collect a maximum amount of data. To also find species which are only recognizable in early Spring or in late Autumn, several visits are crucial. As a rule, it can be stated that an area should be visited at least two times, *e.g.,* in the lowlands of Northern and Central Europe the best time for surveying the flora is in Spring and Summer, in the Mediterranean region in early Winter and late Spring, in tropical regions prior to and immediately after the rainy season. The timing of fieldwork is further dependent on the sea level of the investigation area, on predominant habitats, on the substrate, and on the local (micro)climate.

Knowledge of local experts and the study of literature and herbarium vouchers help to choose the best time, but be aware of overall weather conditions in the year when the investigation takes place. The weather influences highly the phenology of plants (*e.g.*, Pfeifer, 1996). Very hot weather accelerates the growth and flowering of plants and cold weather may retard growth by up to four weeks or more. In deserts, the majority of vascular plants are annuals which germinate and flower only after rainfall. Precipitation, and thus these annuals, may not occur for several consecutive years.

2.3. Fieldwork design

Once the aim of the fieldwork and the target area has been chosen, the method of recording data must be selected. There is no method, which is suited to every inventory or investigation region so the influence of the chosen method of sample

design, *e.g.,* the size of grids or the size, position and even the shape of sample plots (Keeley & Fotheringham, 2005) on results should be remembered.

It must be emphasized that searching, recording, and mapping taxa in a given area or region is distinct from qualitative vegetation analysis where a subjective, rather than a non-random or systematic, selection may be regarded as problematic (Daubenmire, 1968; Müller-Dombois & Ellenberg, 1974). In fact, in order to record all species, including the rarest, the selection of sample sites and transects, respectively, should not be done in a systematic or random way, but should be adapted to the heterogeneity of the terrain and the types of vegetation, respectively. Furthermore, a complete inventory requires careful attention to all microhabitats and transitions of plant communities. To record a maximum percentage of taxa in an area, all vegetation types and especially habitat borders should be visited: *e.g.* dunes, shingles, cliffs, inland surface waters, mires, bogs, fens, grasslands, forb vegetation, scrubs, heaths, woodland, forests, ruderal places, agricultural and artificial habitats. Tree falls are valuable sources of branches with leaves, flowers, and fruits as well as epiphytic and liana vegetation which are usually not easily accessible.

The flora of a small region may be surveyed completely by covering the whole area and surveying all taxa within this area. Larger areas are usually divided into grids, the flora of each grid being surveyed separately (see below). In the case that an area is too large for a complete exploration or else if personal, temporal or financial resources are too scarce, sample plots are assumed to represent the flora of the whole region. Before fieldwork takes place it must be decided whether and how many single scale plots, transects or nested multiscale plots are chosen. The number of plots necessary to record plant diversity most accurately strongly depends on the diversity of habitats and on the homogeneity of vegetation and must be defined in view of including all habitats and may include replications. As a rule, one has to find the balance between the completeness of the taxa inventory and time- and cost-efficiency. For benefits and drawbacks of several field methods see Rich *et al.* (2005) and Stohlgren (2006), for the tropics in particular Dallmeier (1992) or Jermy & Chapman (2002).

Data should be collected in a way that is traceable in the study area years later and fit for monitoring purposes. In order to increase efficiency and to allow accurate replications of methods fieldtrips should be well documented, *e.g.,* the number and experience of the staff involved, the time spent in the field and logistics of the fieldwork. Photographs of the sites may be helpful for monitoring purposes, provided that they contain permanent field markers, *e.g.* trees, buildings, prominent rock formations, in such a way as to easily understand the position of the photographer. Alternatively or in addition, the position of the photographer as well as the direction of the shooting should be recorded. The scale of maps used in the field should be at least 1:50.000, optimally 1:25.000 and in large areas with a homogenous flora maximally 1:100.000.

When selecting sample plots one should consider also the susceptibility of the terrain to trampling damage caused by fieldwork. If such damages are expected, access must be limited. As to the sensitivity of habitats in general, an appeal is made to common sense.

2.3.1. Flora mapping of grid cells

A widespread method for surveying plant diversity in a region is constituted by the flora mapping of grid cells whose size and position is given by the mapping project or conform to the grids used in the region (*e.g.,* UTM, 'quadrants'). Grid cells are either explored exhaustively or the flora of each cell is recorded in a representative manner by means of excursions following a fixed pathway. The results for each region and grid cell, respectively, are shown in the form of a checklist. Mapping grid cells is highly recommended. In fact, since all cells have to be explored regardless of possible logistical obstacles or the mappers' laziness, this kind of mapping provides a differentiated picture of the distribution of species in the study area. It is recommended that the investigation area is divided into grid cells which can be investigated within a day or half a day.

2.3.2. Single sample plots

Generally, the size and number of sample plots has to be adapted to the given vegetation. Several methods are available to determine the minimum size of a plot for recording a pre-assigned (high) percentage of species in different vegetation types. Best known is the 'minimum area' method used in phytosociology. It has fundamentally influenced the determination of sample-plot size (see bibliography of Tüxen, 1970; Barkman, 1989; Dietvorst *et al.,* 1982). Other, similar methods include the calculation of species accumulation curves (*e.g.,* Fisher *et al.,* 1943; Barbour *et al.,* 1980; Palmer, 1990; Palmer *et al.,* 1991; Elzinga *et al.* 1998; see also the discussion in Chong & Stohlgren 2007, Hui 2008; Gray *et al.,* 2004a, b; Keeley, 2003; Scheiner, 2003, 2004) but in the context of the fieldwork they seem rather elaborate and time consuming. Furthermore, they do not necessarily account for the presence of rare species sufficiently. Therefore, it is preferred to use empirical values which are applicable in the field (Table 1). However, in regions with an estimated rich but unknown flora, plot size determination by means of statistical methods is highly recommended. The plots were measured off in the field using tape and marked with ground stakes, coloured bands and/or small flags.

2.3.3. Transects

The transect method is recommended for large areas with one or more ecological gradient *e.g.,* humidity, sun exposition, edaphic conditions or altitude. To inventory for all taxa, all vegetation types must be considered. To set a transect means to define a plot, usually of a (long) rectangle shape, within an area comprising the ecological gradients. By doing so, the maximum range of habitat and species diversity can be covered within a minimum space and with a minimum of resources. Transect length and width largely depend on the size of the investigation area. If a transect is large, sample plots may be defined within the transect at regular distances. Transect sample distances will depend on vegetation uniformity and the overall transect size.

Vegetation types outside but in the immediate vicinity of the transect should also be investigated for new taxa but the records kept separately. For a usable

transect method in tropical forests along a precipitation and latitudinal gradient see, *e.g.,* Gentry (1982, 1995) or Clinebell *et al*. (1995).

	Müller-Dombois & Ellenberg (1974)	Dierschke (1994)
Rock vegetation, spring meadow vegetation,		up to 5 m²
Fens, pioneer lawn, and pastures		up to 10 m²
Herbs	1-2 m²	
Coast dunes, wet and dry meadows, mountain meadows, heath, bulky sedges		10-25 m²
Dry-grassland	50-100 m²	
Weed and ruderal vegetation, scrubs, rocky meadows		25-100 m²
Tall herbs-low shrubs	10-25 m²	
Tall shrubs	16 m²	
Large plants/trees/forest	200-500 m²	>100 - >1000 m²
Forest understory only	50-200 m²	100-200 m²

Table 1. Adequate single plot sizes for flora and vegetation analyses.

2.3.4. Multiscale plots

Instead of using several smaller sample plots or few large transects, multiscale plots as overlaying nested quadrats of increasing size (*e.g*., Müller-Dombois & Ellenberg, 1974; Barnett & Stohlgren, 2003) can be used. Among them, the modified Whittaker plot (Whittaker, 1977; Shmida, 1984; Stohlgren *et al.*, 1995) has proven itself in practice. The modified Whittaker plot is a combination of one 1000 m² plot containing subplots of several sizes (Fig. 1). While the flora of the smaller plots is recorded exhaustively, less extensive systematic surveys are carried out in the larger plots. This design has been increasingly applied in the last years for the calculation of plant diversity (*e.g*., Keeley *et al.*, 1995; Bellehumeur & Legendre, 1998; Carrington & Keeley, 1999; Brown & Peet, 2003; Byers & Noonberg, 2003; Bruno *et al.*, 2004; Fridley *et al.*, 2004; Davies *et al.*, 2005). Multiscale-sampling is more labour- and cost-intensive but it allows estimates of species richness and plant diversity patterns to be made. This approach is based on the assumption that patterns of plant diversity can be calculated only on the basis of multiscale sample plots (Shmida, 1984). It is particularly helpful if the collected data is statistically evaluated (*e.g.,* for extrapolating species richness or total diversity) and allows diverse plant communities to be compared.

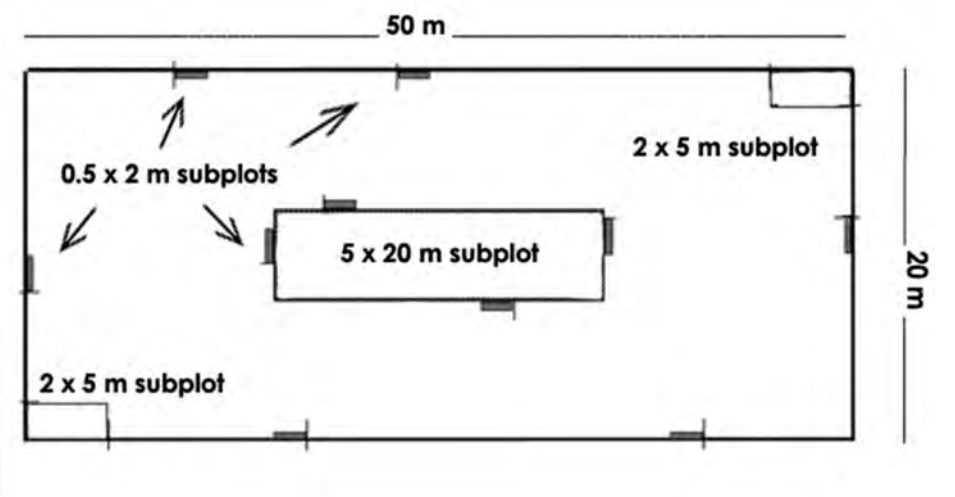


Fig. 1. An example for a modified Whittaker (Shmida, 1984; Stohlgren *et al.,* 1995) plot.

2.3.5. 'Tips and Hints'

For larger, complex areas it is recommended that several fieldtrips are undertaken during different seasons and that each utilises several plot-based-sampling techniques to record a high percentage of the vascular plant flora for checklists and to monitor plant diversity as accurately as possible.

Research can benefit from studying geological maps, biotope maps or high resolution satellite images prior to fieldwork. In fact, this will facilitate the efficient planning and implementation of fieldwork. Possible barriers and dangers in the field, like steep slopes, insurmountable streams or fens (as well as the possible appearance of wild animals) should be identified in the planning phase.

For monitoring plots it is helpful to mark the edges and the centre of each plot with magnets in order to localise the plot later by means of special detectors. Since magnets, particularly when buried several cm into the soil, may get lost, it is recommended that the plots are marked on a map and their coordinates recorded.

2.4. Taxa Recording

To inventory vascular plant taxa is to record all visible taxa – vegetative plants, bloomy plants as well as plants with fruits – by searching the whole area or representative plots for the purpose of compiling or verifying a checklist. A complete inventory includes, of course, not only dominant and frequent species but also rare and inconspicuous ones. In fact, these can make up half of the taxa in a region (Stohlgren *et al.,* 2000) yet are often only recorded after systematic, targeted and time-consuming surveys.

In the field, all plant taxa are to be noted with scientific names. Taxonomy (and preferably also nomenclature) should refer to a widely accepted modern (local)

flora. Exceptions, *e.g.,* if detected species are not (yet) treated in the reference flora or if the field worker adheres to another species concept, should also be documented. Herbarium specimens should be collected for at least those taxa that are: (i) new to the region, (ii) indicated as doubtful, (iii) belonging to taxonomically critical groups (see below). If resources allow, all taxa should be documented by at least one herbarium specimen (see below).

With a few exceptions, *e.g.,* in species-poor habitats with short growing seasons, a species inventory in a certain place and time is hardly ever complete, even when carried out by experienced botanists, and always represents a snap-shot in time. This is because species show different phenology and because the species composition of almost every habitat is subject to ongoing changes. Competent surveyors add significantly to the likelihood of a complete species list as do small survey areas and amply time available for the fieldwork. Likewise, consulting regional floras prior to the fieldwork will give an estimate of the species number to be expected, and provide a comparative list to evaluate the field results against. Statistical methods for evaluating the completeness of the taxa inventory are provided by, *e.g.*, Heltshe & Forrester (1983), Miller & Wiegert (1989), Palmer (1990), Palmer *et al.* (1991).

2.4.1. Providing additional data and metadata

The quality of biodiversity data depends on the calibre and quantity of additional data and metadata provided. Parameters include constant ones, among them mainly geographic data (see above), as well as those which are to be recorded at each collecting date and which have a considerable impact on long-term changes in plant diversity: biotic data concerning, *e.g.*, phenology or herbivory, and abiotic data concerning disturbances caused by extreme atmospheric conditions, fire, windstorms, geological processes or human impact. This is also important for monitoring. The dynamics of the populations in an area can be observed in detail over the period of monitoring more effectively if larger numbers of parameters are recorded, *e.g.*, size, extent and vitality or fitness of the population.

Record additional data separately for each region / subregion / plot / transect in a fieldbook (notebook) or on a passport data form. The documentation should include (see also methods and standards on georeferencing):

- Name and address or institution of the field workers.
- Collecting date.
- Location (country, nearest city or landmark described with cardinal direction), exact position and altitude of a record using a map or a Geographical Positioning System (GPS). Reference must be made to the map projection and geodetic datum. Avoid local terms and hints for landmarks and sites which are only known to people who know the locality.
- Ecological conditions (*e.g.,* edaphic conditions, gradient, cardinal direction, trophic level).

- Habitat type (*e.g.,* EUNIS classification), vegetation type, and human use or impact as well as predominant or characteristic species.
- Population size, vitality.

The size of a plant population (*i.e.* all individuals of a species in a region at the same time) which should be recorded wherever possible is highly influenced by environmental conditions, dispersal barriers, and specific breeding system. It is sometimes difficult or even impossible to define and delimit a population; the same holds true for an individual (*e.g*., Silvertown & Charlesworth, 2001; Gibson, 2002; Crawley, 1997; Gurevtich *et al.*, 2003).

Frequently, an exact description of population size makes sense only for clearly delimited populations such as species occurring *e.g.* in small patches of dry grassland, clearings in forests and small raised bogs. The size of a delimited population can be determined by counting or measuring the individuals, visible shoots or the area covered.

In the field, a practicable procedure is recommended and the frequency of the species in the investigation area at least should be assessed through proxy measures such as the number of individuals in samples, individual abundance, the area or through a combination of these *i.e.* the 'cover-abundance' (*'Artmächtigkeit'*) in a sample plot. The disadvantage of estimated values is that they do not represent exact measured data and may differ between field workers. However, experience has shown that they have merit for the description of the flora and vegetation of a region.

2.4.1.1. Distribution in the investigation area

The area covered by a population may serve as the base for monitoring species and populations (Jones, 1998; Brzosko, 2003), and should, in case of small populations and rare species, be estimated as accurate as possible. In the case of larger populations it is useful to map their boundaries if possible, preferably with the help of high resolution satellite or aerial images.

2.4.1.2. Abundance

Recording abundance (*i.e.* the number of individuals of a taxon in a given area) of all species occurring in the investigation area, wherever possible, is recommended. Abundance is a common parameter used to monitor rare plants and small areas. One must bear in mind, however, that recording abundance is often a difficult task insofar as it is sometimes difficult or even impossible to determine what an individual is. In fact, while individuals can easily be recognized in annual or biannual herbs or trees with one stem, this is difficult or impossible in clonal plants. In practice, it has proven useful to refer to shoots and leaf rosettes when counting 'individuals' of clonal, non-flowering or non-fruiting plants. Generally, the abundance of a taxon is recorded through rough estimation of individuals per investigation site, using a logarithmic scale as shown in the example in Table 2 (see also discussion in Barkman *et al.*, 1964). An alternative

is to use simple descriptor such as ‘rare’ or ‘frequent’ which at least give information about the representation of the species in the field.

Abundance class	Abundance in the investigated area / sample plot
1	one individual (very rare)
2	2-10 individuals (rare)
3	11-100 individuals (common)
4	101-1000 individuals (frequent)
5	> 1000 individuals (very frequent)

Table 2. Scale for rough estimation of abundance in a given investigation area or sample plot.

2.4.1.3. Cover

The amount to which plants of a species, seen from the ground (surface), cover a specific area of ground is called ‘cover’. It is often easier to assess cover than abundance, as individuals do not have to be delimited. Estimating cover is particularly useful when dealing with stoloniferous species, among them many Poaceae and Cyperaceae. A frequently used scale for cover estimation (see also Barkman *et al.*, 1964; Braun-Blanquet, 1964) is shown in Table 3.

Cover classes	Range	Midpoint
1	0-5%	2.5%
2	5-10%	7.5%
3	10-25%	17.5%
4	25-50%	37.5%
5	50-75%	62.5%
6	75-100%	87.5%

Table 3. Scale for estimation of cover.

Combined abundance / cover scale

When dealing with small plots, particularly in the framework of monitoring selected rare and endangered species or habitats, a vegetation relevé is recommended using the Braun-Blanquet’s cover-abundance scale (Braun-Blanquet, 1964) modified in the lower scale range by Reichelt & Wilmanns (1973) (Table 4). This is particularly recommended in regions where phytosociological studies, including a syntaxonomical system, have already been carried out. The vegetation relevé requires records to be taken in a specific and comparable

manner. The required records include the flora of the sample plot, the number of individuals (if feasible, see discussion above) and species cover. Furthermore, the method also provides a phytosociological survey. Relevés must correspond to the current phytosociological practice, *i.e.,* they must be based on homogeneous and sufficiently large areas.

scale	combined abundance/cover classes (Artmächtigkeit)	number of individuals
r		1
+		very few
1	0-5 %	variable
1m or 2m	< 5 %	> 100
2a	5-12,5 %	variable
2b	12,5-25 %	variable
3	25-50 %	variable
4	50-75 %	variable
5	75100 %	variable

Table 4. Cover-abundance scale (according to Reichelt & Wilmanns, 1973; Dierschke,1994).

2.4.2. Fitness Parameter

Besides data regarding size and distribution, information concerning the fitness may provide valuable hints about the status of the population. In the framework of mapping projects it is advisable to take into consideration parameters which can be ascertained quickly and easily, for example (approximate) mean height of plants, leaf size (Jones, 1998) or the proportion of flowering and fruiting plants. If monitoring includes revisiting individuals, these need to be adequate marked. Use for example rustproof metal tags fixed to a bar in the ground or fixed on branches. In addition, geo-data must be recorded. Many fitness parameters require time-consuming recording techniques and are generally used only in special monitoring projects. Such parameters include, *e.g.,* leaf size, number of seeds or fruit sets, number of seeds per fruit, germination rate, biomass, development of leaf rosettes and number of flowers (*e.g.*, Brzosko, 2003; Vitt & Havens, 2004; Willi & Fischer, 2005; Janečková *et al.*, 2006).

2.4.3. 'Tips and Hints'

In the field, it is convenient to mark off the observed taxa directly in a checklist of all taxa known from the region. Lists of critical taxa combined with knowledge from local experts point the fieldworker's attention to these taxa. Special

seasonal lists or marking checklists for, *e.g.*, Spring taxa, helps mapping in the beginning of the vegetation period.

If using a checklist to mark the species directly in the field, use one list for each grid, transect or sample plot, respectively. Before switching over to other vegetation types or new areas (*e.g.*, new grid, plot or transect) check carefully the edge of habitats, microhabitats like rocks, and inaccessible sites like the understory of (thorny) shrubs or nettle plants for tiny, prostrate species.

Record all data instantly in the field! After a long collecting trip it is impossible to remember all details.

A passport (collecting) data form is included in the appendix. It can be adapted to personal needs. Checklists and passport forms used for fieldwork should not be copied on white but on coloured or grey recycled paper, because white paper is strongly reflective on sunny days. When getting wet, absorbent paper dries faster than ordinary paper. Leave some blank lines in the fieldbook or data form between two collection notes for additional observations and comments. Bear in mind that someone else might need to read your personal comments, therefore, write legibly using a soft pencil or pen with water resistant ink and avoid any kind of (personal) abbreviation. Once lost in the field coloured notebooks and pens are easier to recover in dense vegetation! Finally, don't forget to backup all your field notes by photocopying the field notebook or the passport sheets as soon as possible.

The use of a dictation machine can be very helpful, especially in bad weather.

2.5. Making herbarium specimens

For species inventory and monitoring in particular, the collection of herbarium specimens is necessary to check field identification, especially when dealing with critical taxa. The high value of herbarium specimens as the basic of botanical research (taxonomy, morphology, phylogeny, ecology, phytosociology, ...) cannot be overemphasized.

In most herbaria, rare taxa (often from only a few well known localities!) are overrepresented, whereas common species are represented by only a few specimens. In order to set up a representative collection in herbaria, however, it is necessary to collect material from frequent and common taxa as well as from infrequent and rare taxa. The value of a herbarium voucher increases significantly with the collector's accuracy when choosing, collecting, pressing, arranging and documenting the voucher. The basic techniques of this procedure are the subject of the next paragraph. For a further in-depth study we refer to literature which offers a comprehensive introduction into the issue (*e.g.*, Savile, 1964; Radford *et al.*, 1974; Jain & Rao, 1977; Cullen, 1984; Lot & Chiang, 1986; Vogel, 1987; Stace, 1989; Walters & Keil, 1996; Bridson & Forman, 2004; Linnartz, 2007).

Numerous plant groups require special collecting techniques. Among these groups are succulent or fleshy plants (*e.g.*, Fosberg & Sachet, 1965; Jain & Rao, 1977; Leuenberger, 1982), aquatic plants (Taylor, 1977; Lot, 1986; Haynes,

1984; Rayna-Roques, 1980), Araceae (Nicolson, 1965; Croat, 1985), Balsaminaceae (Grey-Wilson, 1980), Bromeliaceae (Aguirre León, 1986), Bambusoideae (McClure, 1965; Soderstrom & Young, 1983), Lentibulariaceae (Taylor, 1977), Musaceae (Fosberg & Sachet, 1965), Palmae (Balick, 1989; Dransfield, 1986), Pandanaceae (Stone, 1983), Pteridophyta (Holttum, 1957; Henty, 1976), and Zingiberaceae (Burtt & Smith, 1976).

Beginners and students are urged to visit a herbarium prior to fieldwork. By doing so they may acquaint themselves with the most important features of a herbarium.

2.5.1. Collecting

When collecting herbarium specimens in the field, select individuals representative in size, morphology and colour. Plants should be as complete as possible and include inflorescences, fruits and seeds, as well as all types of leaves (small and large, young and older leaves, ground and stem leaves, rosette leaves, bracts), especially in heterophyllous species, and roots or rhizomes, respectively. Be aware that organs (especially rhizomes) may be cut or broken and thus overlooked easily when digging the plant. Further, keep in mind that some species are dioecious and should be represented in the herbarium by both female and male plants. All other features important for species determination that cannot be drawn from the herbarium specimen, such as stem characters, bark structure and life form, ought to be noted in the field book or the data sheet. Record colours and scents of flowers and leaves, if noteworthy, since these features may vanish or change during pressing or over time. Additionally, photographs of such details may be attached to the herbarium sheet. Avoid collecting untypical small plants solely because they fit the herbarium sheet size. Try to make them fit by using adequate techniques (see below).
When encountering populations which include only a single or few individuals no complete plants must be harvested. The same holds true for very rare and endangered species. If absolutely essential, take a small part of one plant which shows all morphological features necessary for a correct determination. In any case, take photographs of all important details.

If you collect more than one specimen, these should cover the morphological variation within the population. Collect, if possible, plant material enough to produce at least three specimens: one for an institution of the country of origin, one for the species identifier as ‘reward for determination’ and one for your institution. The locations of the duplicates should be documented.

Each specimen should be provided with a unique collection number, *i.e.* a number which, in combination with the collector’s name, unambiguously identifies a specimen. This number can be attached to the specimen with a fixed tag (*e.g.*, jeweller’s tag), labelled with pencil or water resistant ink. Use a serial number sequence which allows for unambiguous identification of all specimens (*e.g.*, Smith, 2340). Prepared tags with running numbers can help handling the vouchers. Numbers of the specimens and pictures, geo-data and detailed documentation must be noted on the collecting sheet or in the field notebook.

Plant samples can be stored in plastic bags or pressed immediately in the field. The advantage of pressing in the field is that the specimens maintain their shape to such an extent that, after the field trip, the position of flowers, stems and leaves can be arranged and corrected without difficulties before drying the specimen. Many taxa (*e.g.,* species of *Linum, Cistus*, *Hibiscus, Impatiens*) have flowers or leaves too delicate to be stored in plastic bags. Specimens of these taxa are best pressed immediately, and some of their flowers put into spirit (see below). To protect delicate flowers, press them in kitchen paper or toilet tissue, this should not be removed until the flowers are completely dry. For the field press, use a DIN A3 or A4 portfolio or two lightweight boards filled with newspaper and a few corrugated cardboards. If plastic bags are used for collecting, use separate bags for small plants and others for large, heavy plants. You can delay wilting by increasing humidity within the bag: put some water in the bag, close it, shake it and remove the surplus of water; too much water may lead to the collapse of flowers and leaves. Transport water plants in water.

Sometimes it is necessary or helpful to put collected plants or parts into chemical fixatives (*e.g*., Tomlinson, 1965). Normally, 70% alcohol is used (in emergencies high proof spirits (*e.g.,* Vodka, Gin, Rum) can be used as a substitute), optionally with a few drops of glycerine. Also common are mixtures of alcohol and glacial ethanoic acid at a ratio of 18:1 (AA) or mixtures of alcohol, formalin and glacial ethanoic acid at a ratio of 18:1:1 (FAA). After the fixation for 2-3 days in AA or FAA, the samples are transferred to 70% ethanol for storage.

In this way, delicate and tender floral characteristics relevant for a correct identification can be preserved. This is particularly important for taxa in the Aristolochiaceae, Asclepiadaceae, Balsaminaceae, Begoniaceae, Commelinaceae, Gesneriaceae, Lentibulariaceae, Orchidaceae, Orobanchaceae, Passifloraceae, and Portulacaceae. In case of tender water species plants may be fixed as a whole, in case of Gymnospermae with easily dropping needles (*e.g*., *Picea*, *Tsuga*) whole branches may be fixed.

The hermetically sealed tubes or bottles with the fixed plants should be labelled (small labels, pencil!) inside and outside, and the cap of the container should additionally be wrapped in Parafilm.

When collecting herbarium specimens, it is easy to collect silica gel samples for DNA-banks or/and seeds simultaneously (ENSCONET, 2009).

2.5.2. Pressing

Place each specimen in a newspaper sheet or between very thin, yet strong absorbent paper and arrange it as carefully as possible. Spread the leaves in such a way as to not cover the stem, flower and fruits. Leaves should overlap as little as possible. Reverse at least one leaf, in order to make both sides visible when the specimen is mounted on a herbarium sheet. Ensure all leaves are smoothly pressed. Make sure that flowers are arranged in different positions so as to make visible the calyx, stamens and carpel. Divide the flowers or cut dense inflorescences, like the capitulum of Asteraceae, in order to reveal hidden bracts. In the same way cut large fruits or thick stems.

Overlapped parts of the plant should be separated with tissue paper. If branches are too thick leaves and flowers get pressed insufficiently and become wizened. In such cases the empty space between (thinner) organs and hardboard may be filled with tissue paper so that all plant parts undergo the same pressure. If the plant is too big to fit into the press, fold the stem and big leaves, or divide the plant and press the single parts in different folders.

Palm leaves should be cut round the hastula, *i.e.* the leaf base, which is important for species identification, and further features of the palm leaves like size or the position of the inflorescence should be noted. Leaves of big ferns should be divided: press apical, mid and basal parts, and the petiole separately. Note the arrangement of the pinnae and the leaf size (Holttum, 1957; Henty, 1976).

Succulent and fleshy plants need a special pressing and drying procedure. Cut the plants and kill them by putting the parts either into boiling water, in the microwave or in alcohol (Fosberg & Sachet, 1965; Leuenberger, 1982; Womersley, 1981).

Aquatic plants need a special treatment, too (Taylor, 1977; Lot, 1986; Rayna-Roques, 1989). Arrange them on a paper floating in a tub filled with water, the paper being of the same size of the definite herbarium sheet. After the arrangement pour the water slowly and carefully out of the tub. The plant will remain attached to the paper sheet and is ready to undergo the regular drying procedure (see above).

Fig. 2. Simple equipment for pressing plants: plywood pieces or metal frames for the outsides of the press, absorbent paper, corrugated cardboard, and lashing straps.

Fig. 3. Plant press, with specimens in newspaper sheets between corrugated cardboard.

Between the papers with the specimen, put blotting paper or corrugated cardboard. Place this stack between two light boards with holes for better drying and clamp it securely with two or three straps (Figs 2 & 3).

2.5.3. The Alcohol or 'Schweinfurth' press

Sometimes, especially in the Tropics, drying equipment is not available. In such cases the use of the alcohol press (Womersley, 1981) is recommended. To conserve your collection with alcohol, bundle the newspaper with the specimen and put it into leak proof plastic bags. Make sure that the specimens are labelled with alcohol resistant ink (black china) or a soft pencil. For a pack with a high of 20 cm you need about 1 litre of 50-70% ethanol or isopropanol. Pour alcohol into the bag, turn the bag several times to disperse the alcohol and store the bundle in a horizontal position. Turn it every day until the bundle is completely saturated with alcohol. Avoid too much solution: the bundle must be completely moist, but not wet. After arriving in the lab or herbarium, dry the specimens in a drying oven as if they were fresh material. Treating the press with highly toxic formalin solutions should be avoided for environmental reasons.

The advantage of this method is that the specimens are protected against mould, but there are several disadvantages: the plants loose their colour, the specimen becomes brittle and it cannot be used as a source of DNA.

2.5.4. Drying

The faster the drying process the better the specimen will be conserved. Keep the press in a well aired warm place; if possible, expose it to the sun. If no drying sets are available, the drying paper or corrugated cardboard layered between the specimens need to be replaced every day within the first couple of days (depending on the plant material). At the first change, the correct arrangement of the whole plant must be checked, especially when dealing with delicate flowers and leaves. If the plants are very wet, replace the drying paper after three to four hours. Later, changing the paper is only necessary every second or third day until the specimens are completely dry. Coriaceous leaves need a lot of time to dry and may appear dried though still wet. To test whether they are dry bend the leaves carefully: if they are still twistable leave them in the press to continue drying.

Under humid conditions as in the tropics a drying set is recommended. Such a set is based upon air-drying forced by a fan heater or other heat sources. The warm air is conducted through the plant press, thereby drying the plant material. Botanists have competed with each other to invent (funny) drying constructions by using various heat sources like charcoal, light bulbs, kerosene or propane (which doesn't work in high altitudes due to the low oxygen content of the air!). However, exaggerated heating is to be avoided to preserve colours and to prevent browning of plant tissue (Camp, 1946; Allard, 1951).

Fig. 4. Drying set with an electric heater and a funnel of fire resistant canvas.

We suggest a simple and cheap technique by using a small electric heater. Wherever electricity is available this is a safe and quick way to dry plants. Connect the heater and the press with a funnel of fire resistant textile, *e.g.*

canvas which you can sew in the exact size of heater and press (Fig. 4). Put up to four newspaper folders containing the specimens between two corrugated boards. Piled in this way, the whole pack can be dried overnight. Pay attention that the corrugated cardboard is arranged longitudinally to the airflow and metal framed plant presses are not used.

It is also possible to dry plants with an iron by wrapping them in highly absorbent drying paper and ironing with low temperature and moderate pressure. Replace the paper when it becomes moist. Ironing with temperatures of around 30°C (but not more!) permits drying of delicate flowers and preserves colours. It is not recommendable to use an oven for plant drying because in an oven there is no exchange of air. If an oven is the only source of heat, make sure that the warm air flows through the corrugated cardboards.

If external heat sources are not available, silica gel may be used for plant drying instead. For that purpose the press, which should not be too huge, is put into an air permeable fabric bag. The bag is then placed together with silica gel inside an airtight plastic bag. The silica gel will have to be changed more often if the plants are very wet or there is only a small volume of silica gel. Indicator silica gel which changes colour when saturated with water is recommended. Silica gel can be dried in an oven and used repeatedly.

If drying systems provided with external heat sources are used, be aware of fire, especially when handling specimens conserved in alcohol! Inside of buildings do not forget to install a fire alarm in your room.

2.5.5. Herbarium sheets

Each specimen is provided with a herbarium label containing at least the following standard information: collection site including exact description of the locality (state, province, district, toponym), coordinates, altitude and information regarding the habitat (*e.g*. surrounding vegetation); the collector's name; collection date. At best, additional information may appear on the label for example the chorological status (if known or estimable) or noteworthy observations regarding *e.g.* population size, threat, …

Usually specimens are mounted on a white cardboard paper by means of gummed paper stripes or glue from hot-glue guns. Seeds and other small broken plant parts are normally stored in paper capsules which are attached to the herbarium sheet. As each large big herbarium has its own standards and methods of moulting this topic will not be covered further in this manual. See special literature (*e.g.,* Bridson & Forman, 2004; Liesner, 2009) and study label examples (Fig. 5) for that purpose.

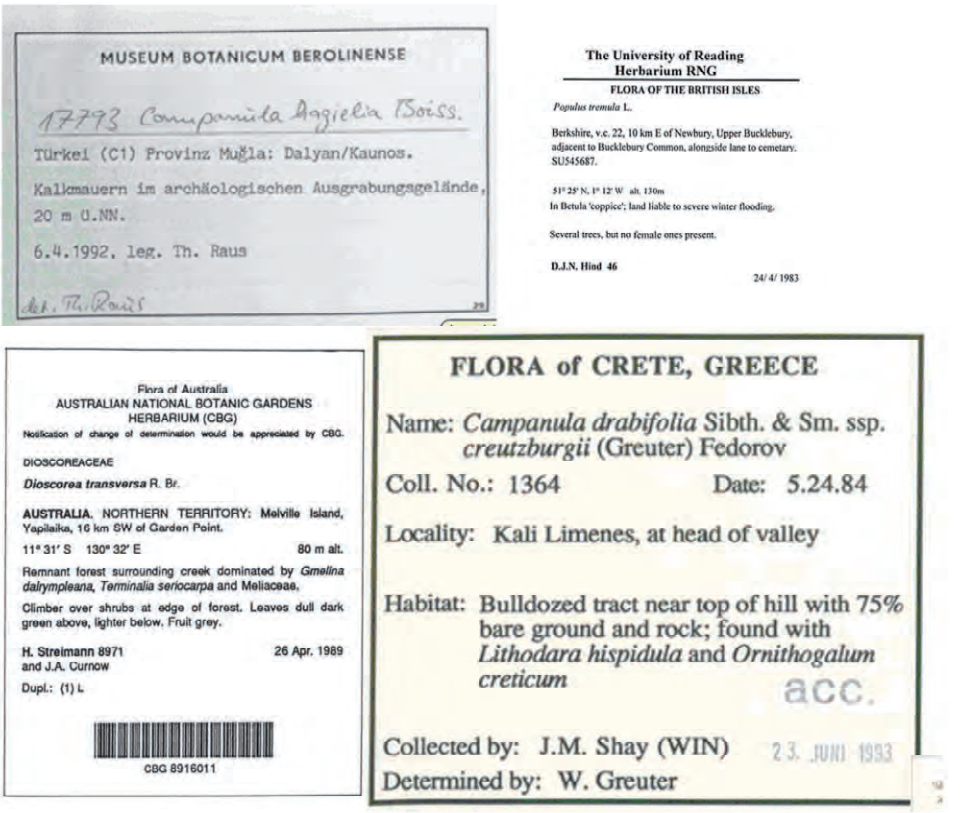


Fig. 5. Examples of herbarium specimen labels.

2.5.6. 'Tips and Hints'

Be aware of poisonous species, or plants with stinging hairs, thorns and prickles especially if you are not familiar with the regional flora, *e.g*. in the tropics!
Collect only as many plants as you can process in a day! A collection of a few well documented and preserved specimens is far more useful than a large quantity of bad and fragmentary specimens with incomplete and doubtful documentation. If it is not possible to press all plants collected in a day, store robust plants *e.g.*, succulent or lauriphyllous species in a cool moist place (*e.g*. in the fridge) overnight.

Supply yourself with newspaper whenever possible, *i.e.* before and during the field trip. The quantity of paper required is considerable!

Any kind of transport represents a serious risk of damaging the collected plant material. Wrap specimen bundles tightly to prevent mechanical damage, *e.g.* during postage. In case of long-distance shipping a treatment with insecticides may be necessary.

After the drying procedure it is recommended that the collected plant material is put in a freezer for three days at least to kill insects (including all their developmental stages) and to avoid contamination to other collections.

3. Conclusions

Recording all higher plant species of a given region is a complex task, which ought to be planned carefully. Even when satisfying scientific criteria during field work, we must bear in mind that the results of our survey always reflect reality only for a given moment in time.

The first thing to do, when carrying out a taxa inventory, is to gain a general idea of the study area and check whether any floristic data is already available. The recording itself may be accomplished either through a complete survey over the whole area or through a survey of representative plots and results in a species list. Providing additional, population specific and ecological data with the species list increases the value of the final checklist. As does an accompanying collection of representative herbarium specimens. Fieldwork should be well documented. The more (detailed) data are recorded the more valuable and significant they are and the greater the solid base for subsequent monitoring projects. It appears more reasonable to survey the flora of a limited (small) area by providing comprehensive and detailed data rather than to deal with a large area by yielding incomplete and poorly documented results.

Observing nature attentively in the field means, on the one hand, learning to understand fascinating ecological interactions and, on the other hand, experiencing the beauty and quality of nature.

4. References

AGUIRRE LEÓN, E. 1986. Epífitas. *In*: LOT, A. & CHIANG, F. (Eds). *Manual de herbario, Administractión y manejo de colecciones técnicas de recolección y preparación de ejemplares botánicos*. Consejo Nacional de la Flora de México, A.C. México: 113-119.

ALLARD, H.A. 1951. Drying herbarium specimens slowly or rapidly. *Castanea* 16: 129-134.

ASHTON, P.S. 1998. A Global Network of Plots for Understanding Tree Species Diversity, in Tropical Forests. *In*: DALLMEIER, F. & COMISKEY, J.A. (Eds). *Forest Biodiversity Research, Monitoring and Modeling: Conceptual Background and Old World Case Studies*. Parthenon Publishing Group, Paris: 47-62.

BALICK, M.J. 1989. Collecting and preparing palm specimen. *In*: CAMPBELL, D.G. & HAMMOND, H.D. (Eds). *Floristic inventory of tropical countries*. New York Botanical Garden, New York: 482-483.

BARBOUR, M.G., BURK, J.H. & PITTS, W.D. 1980. *Terrestrial Plant Ecology*. Benjamin/Cummings, Menlo Park CA: 382 pp.

BARBOUR, M.G., BURK, J.H., PITTS, W.D., GILLIAM, F.S. & SCHWARTZ, M.W. 1999. *Terrestrial Plant Ecology*, 3rd ed. Benjamin/Cummings, Menlo Park CA: 688 pp.

BARKMAN, J.J. 1989. A critical evaluation of minimum area concepts. *Vegetatio* 85: 105-116.

BARKMAN, J.J. DOING, H. & SEGAL, S. 1964. Kritische Bemerkungen und Vorschläge zur quantitativen Vegetationsanalyse. *Acta Botanica Neerlandica* 13: 394-419.

BARNETT, D.T. & STOHLGREN, T.J. 2003. A nested-intensity design for surveying plant diversity. *Biodiversity and Conservation* 12: 255-278.

BELLEHUMEUR, C. & LEGENDRE, P. 1998. Multiscale sources of variation in ecological variables: modelling spatial dispersion, elaborating sampling designs. *Landscape Ecology* 13: 15-25.

BERGMEIER, E. 1992. Grundlagen und Methoden Floristischer Kartierungen in Deutschland. *Floristische Rundbriefe Beiheft*. 2: 1-146.

BIRNIE, R.V., TUCKER, G. & FASHAM, M. 2005. General habitat survey and monitoring methods. *In*: HILL, D., FASHAM, M., TUCKER.G., SHEWRY, M. & SHAW, P. (Eds). *Handbook of Biodiversity Methods. Survey, Evaluation and Monitoring*. Cambridge University Press, Cambridge: 154-194.

BONHAM, C.D. 1989. *Measurements for Terrestrial Vegetation*. John Wiley & Sons, New York: 352 pp.

BRAUN-BLANQUET, J. 1964. *Pflanzensoziologie*. Springer, Wien: 865 pp.

BRIDSON, D. & FORMAN, L. 2004. *The herbarium handbook*. 3rd ed. Royal Botanic Gardens, Kew: 346 pp.

BRUNO, J.F., KENNEDY, C.W., RAND, T.A. & GRANT, M.B. 2004. Landscape-scale patterns of biological invasions in shoreline plant communities. *Oikos* 107: 531-540.

BRZOSKO, E. 2003. The dynamics of island populations of *Platanthera bifolia* in the Biebrza National Park (NE Poland). *Annales Botanici Fennici* 40: 243-253.

BURT, B.L. & SMITH, R.M. 1976. Notes on the collection of Zingiberaceae. *Flora Malesiana Bulletin* 29: 2599-2601.

BYERS, J.E. & NOONBURG, E.G. 2003. Scale dependent effects of biotic resistance to biological invasion. *Ecology* 84: 1428-1433.

CAMP, W.H. 1946. On the use of artificial heat in the preparation of herbarium specimens. *Bulletin of the Torrey Botanical Club* 73: 235-243.

CAMPBELL, S.P., CLARK, J.A., CRAMPTON, L.H., GUERRY, A.D., HATCH, L.T., HOSSEINI, P.R., LAWLER, J.J. & O'CONNOR, R.J. 2002. An assessment of monitoring efforts in endangered species recovery plans. *Ecological Applications* 12: 674-681.

CARRINGTON, M.E. & KEELEY, J.E. 1999. Comparison of postfire seedling establishment between scrub communities in mediterranean- and non–mediterranean-climate ecosystems. *Journal of Ecology* 87: 1025-1036.

CHONG, G.W. & STOHLGREN, T.J. 2007. Species–area curves indicate the importance of habitats' contributions to regional biodiversity. *Ecological Indicators* 7: 387-395.

CLELAND, E.E., CHUINE, I., MENZEL, A., MOONEY, H.A. & SCHWARTZ, M.D. 2007. Shifting plant phenology in response to global change. *Trends in Ecology & Evolution* 22: 357-365.

CLINEBELL, R.R., PHILLIPS, O.L., GENTRY, A.H., STARK, N. & ZUURING, N. 1995. Prediction of Neotropical tree and liana species richness from soil and climatic data. *Biodiversity Conservation* 4: 56-90.

CRAWLEY, M. 1997. *Plant Ecology*. Blackwell, Oxford: 736 pp.

CROAT, T.B. 1985. Collecting and preparing specimens of Araceae. *Annals of the Missouri Botanical Garden* 72: 252-258.

CULLEN, J. 1984. Libraries and Herbaria. *In*: HEYWOOD, V.H. & MOORE, D.M. (Eds). *Current Concepts in Plant Taxonomy*. Academic Press, London: 25-38.

DALLMEIER, F. & COMISKEY, J.A. 1996. From the forest to the user: a methodology update. *In*: WILSON, D.E. & SANDOVAL, A. (Eds). *Manu: The Biodiversity of Southeastern Peru; La Biodiversidad del sureste del Peru*. Smithsonian Institution Press, Washington DC: 41-56.

DALLMEIER, F. 1992. Long-term monitoring of biological diversity in tropical forest areas: Methods for establishment and inventory of permanent plots. *MAB Digest* 11. UNESCO, Paris: 72 pp.

DAUBENMIRE, R.F. 1968. *Plant Communities: A Textbook of Plant Synecology*. Harper and Row, New York: 300 pp.

DAVIES, K.E., CHESSON, P., HARRISON, S., INOUYE, B.D., MELBOURNE, B.A. & RICE, K.J. 2005. Spatial heterogeneity explains the scale dependence of the native-exotic diversity relationship. *Ecology* 86: 1602-1610.

DIERSCHKE, H. 1994. *Pflanzensoziologie. Grundlagen und Methoden*. Ulmer, Stuttgart: 683 pp.

DIETVORST, P., VAN DER MAAREL, E. & VAN DER PUTTEN, H. 1982. A new approach to the minimum area of a plant community. *Vegetatio* 50:77-91.

DRANSFIELD, J. 1986. A guide to collecting palms. *Annals of the Missouri Botanical Garden* 73: 166-176.

ELLENBERG, F., HAEUPLER, H. & HAMANN, U. 1968. Arbeitsanleitung für die Kartierung der Flora Mitteleuropas (Ausgabe für die Bundesrepublik Deutschland). *Mitteilungen der Floristisch- Soziologischen Arbeitsgemeinschaft N. F.* 13: 284-296.

ELZINGA, C.L., SALZER, D.W. & WILLOUGHBY, J.W. 1998. Measuring and Monitoring Plant Populations. Report BLM/RS/ST-98/005+1730. U.S. Department of Land Management, Denver CO: 477 pp.

ENSCONET 2009. ENSCONET seed collecting manual. European Seed Conservation Network. Available on http://www.ensconet.eu.

FOSBERG, F.R. & SACHET, M.H. 1965. Manual for tropic herbaria. *Regnum Vegetabile* 39. International Association of Plant Taxonomy, Utrecht: 132 pp.

GENTRY, A.H. 1982. Patterns of Neotropical plant species diversity. *Evolutionary Biology* 15: 1-84.

GENTRY, A.H. 1995. Diversity and Floristic Composition of Neotropical Dry Forests. *In*: BULLOCK, S.H., MOONEY, H.A. & MEDINA, E. (Eds). *Seasonally Dry Tropical Forests.* Cambridge University Press, New York: 146-194.

GIBSON, D.J. 2002. *Methods in Comparative Plant Population Ecology*. Oxford University Press, Oxford: 352 pp.

GOETHART, J.W.C. & JONGMANS, W.J. 1902-1907. *Planten-Kaartjes voor Nederland*. Brill, Leiden: 237 pp.

GRAY, J.S., UGLAND, K.I. & LAMBSHEAD, J. 2004. Species accumulation and species area curves - a comment on Scheiner (2003). *Global Ecology and* Biogeography 13 : 473-476.

GREY-WILSON, C. 1980. Notes on collecting *Impatiens*. *Flora Malesiana Bulletin* 33: 3435-3436.

GUREVTICH, J., SCHEINER, S.M. & FOX, G. 2003. *The Ecology of Plants*. Sinauer Associates, Sunderland MA: 523 pp.

HAYNES, R.R. 1984. Techniques for collecting aquatic and marsh plants. *Annals of the Missouri Botanical Garden* 71: 229-231.

HELTSHE, J.F. & FORRESTER, N.E. 1983. Estimating species richness using the jackknife procedure. *Biometrics* 39: 1-12.

HENTY, E.E. 1976. Notes on the collection of ferns. *In*: WOMERSLEY, J.S. (Ed.). *Plant collecting and herbarium development*. FAO Plant productions and protection paper 33. XI. Rome: 71 pp.

HICKS, A.J. & HICKS, P.M. 1978. A selected bibliography of plant collection and herbarium curation. *Taxon* 27: 63-99.

HILL, D., FASHAM, M., TUCKER.G., SHEWRY, M. & SHAW, P. (Eds) 2005. *Handbook of Biodiversity Methods. Survey, Evaluation and Monitoring*. Cambridge University Press, Cambridge: 588 pp.

HOLTTUM, R.E. 1957. Instructions for collecting tree ferns. *Flora Malesiana Bulletin* 13: 567.

HOMMEL, P.W. & REULER, H. VAN 1989. Forest types of Ujung Kulon (West Java, Indonesia). *Studies in Plant Ecology* 18: 113-116.

HUI, C. 2008. On species-area and species accumulation curves: A comment on Chong and Stohlgren's index. *Ecological Indicators* 8: 327-329.

JAIN, S.K. & RAO, R.R. 1977. A handbook of field and herbarium methods. Today & Tomorrow's Printers and Publishers, New Delhi: 157 pp.

JANEČKOVÁ, P., WOTAVOVÁ, K., SCHÖDELBAUEROVÁ, I., JERSÁKOVÁ, J. & KINDLMANN, P. 2006. Relative effects of management and environmental conditions on performance and survival of populations of a terrestrial orchid, *Dactylorhiza majalis*. *Biological Conservation* 129: 40-49.

JERMY, C. & CHAPMAN, R. 2002. *Tropical Forest Expeditions*. 5th ed. Advisory Centre RGS-IBG Expedition, London: 198 pp.

JNCC 2004. *Common Standards Monitoring Guidance for Vascular Plant Species*. JNCC (www.jncc.gov.uk/pdf/CSM_vascular_plants.pdf).

KEELEY, J.E. & FOTHERINGHAM, C.J. 2005. Plot shape effects on plant species diversity measurements. *Journal of Vegetation Science* 16: 249-256.

KEELEY, J.E. 2003. Relating species abundance distributions to species-area curves in two Mediterranean-type shrublands. *Diversity and Distributions* 9: 253-259.

KEELEY, J.E., CARRINGTON, M. & TRNKA, S. 1995. Overview of management issues raised by the 1993 wildfires in southern California. *In*: KEELEY, J.E. & SCOTT, T. (Eds). *Brushfires in California: Ecology and Resource Management*. International Association of Wildland Fire, Fairfield WA: 83-89.

KREBS, C.J. 1999. *Ecological Methodology*. Benjamin/Cummings, Menlo Park CA: 426 pp.

KÜCHLER, A.W. (Ed.). 1988. *Vegetation mapping*. Kluwer, Dordrecht: 635 pp.

KULL, T., SAMMUL, M., KULL, K., LANNO, K., TALI, K., GRUBER, B., SCHMELLER, D.S. & HENLE, K. 2008. Necessity and reality of monitoring threatened European vascular plants. *Biodiversity and Conservation* 17: 3383-3402.

LEUENBERGER, B.E. 1982. Microwaves: a modern aid in preparing herbarium specimens of succulents. *Cactus and Succulent Journal of Great Britain* 44: 42-43.

LIESNER, R. 2009. *Field Techniques Used by Missouri Botanical Garden*. http://www.mobot.org/MOBOT/molib/fieldtechbook/welcome.shtml.

LINNARTZ, S. 2007. *Die botanische Exkursion - Schritt für Schritt zum eigenen Herbarium*. Quelle & Meyer, Wiebelsheim: 141 pp.

LOT, A. & CHIANG, F. 1986. *Manual de herbario, administración y manejo de colecciones técnicas de recolección y preparación de ejemplares botánicos*. Consejo Nacional de la Flora de México, México: 142 pp.

LOT, A. 1986. Acuáticas vasculares. *In*: Lot, A. & Chiang, F. (Eds). *Manual de herbario, Administración y manejo de colecciones técnicas de recolección y preparación de ejemplares botánicos*. Consejo Nacional de la Flora de México, México: 87-92.

MAGURRAN, A.E. 1988. *Ecological Diversity and Its Measurement*. Princeton University Press, Princeton NJ: 192 pp.

MCCLURE, F.A. 1965. Suggestions on how to collect Bamboos. *In*: FOSBERG, F.R. & SACHET, M.H. (Eds). *Manual for tropic herbaria*. *Regnum Vegetabile* 39. International Association of Plant Taxonomy, Utrecht: 120-122.

MILLER, R.I. & WIEGERT, R.G. 1989. Documenting completeness, species-area relations, and the species-abundance distribution of a regional flora. *Ecology* 70: 16-22.

MÜLLER-DOMBOIS, D. & ELLENBERG, H. 1974. *Aims and Methods of Vegetation Ecology*. John Wiley & Sons, New York: 547 pp.

NICOLSON, D.H. 1965. Collecting Araceae. *In*: FOSBERG, F.R. & SACHET, M.H. (Eds). *Manual for tropic herbaria*. *Regnum Vegetabile* 39. International Association of Plant Taxonomy, Utrecht: 123-126.

NIKLFELD, H. 1971. Bericht über die Kartierung der Flora Mitteleuropas. *Taxon* 20: 545-571.

NOSS, R.F. 1990. Indicators for monitoring biodiversity: a hierarchical approach. *Conservation Biology* 4: 355-364.

NUSSER, S.M. & GOEBEL, J.J. 1997. The National Resources Inventory: a long-term multi-resource monitoring programme. *Environmental and Ecological Systematics* 4: 181-204.

ØKLAND, R.H. 1990. *Vegetation ecology. Theory, methods and applications with reference to Fennoscandia*. *Sommerfeltia* Suppl. 1. Økland, Oslo: 233 pp.

OSTENFELD, C.H. 1931. The distribution within Denmark of the higher plants. I. A brief historical survey of the investigation. Det Kongelige Danske Videnskabernes Selskabs Skrifter, Naturvidenskabelig og Mathematisk Afd., 9. *Række* 3: 1-15.

PALMER, C.J., RITTERS, K.H, STRICKLAND, J., CASSELL, D.C., BYERS, G.E., PAPP, M.L. & LIFF, C.I. 1991. *Monitoring and Research Strategy for Forests-Environmental Monitoring and Assessment Program (EMAP).* EPA/600/4–91/012. U.S. Environmental Protection Agency, Washington DC: 192 pp.

PALMER, M.W. 1990. The estimation of species richness by extrapolation. *Ecology* 71: 1195-1198.

PEREIRA, H.M. & COOPER, H. 2006. Towards the global monitoring of biodiversity change. *Trends in Ecology & Evolution* 21: 123-129.

PERRING, F.H. & WALTERS, M. (Eds). 1962. *Atlas of the British Flora.* Botanical Society of the British Isles, London: 432 pp (2d ed. 1975).

PETERS, R.L. & LOVEJOY, T.E. (Eds). 1992. *Global Warming and Biological Diversity*. Yale University Press, New Haven CT: 408 pp.

PFEIFER, M., WIEGAND, K., HEINRICH, W. & JETSCHKE, G. 2006. Long-term demographic fluctuations in an orchid species driven by weather: implications for conservation planning. *Journal of Applied Ecology* 43: 313-324.

PLACHTER, H., BERNOTAT, D., MÜSSNER, R. & RIECKEN, U. (Eds). 2002. Entwicklung und Festlegung von Methodenstandards im Naturschutz. *Schriftenreihe für Naturschutz und Landschaftspflege* 70: 1-564.

RADFORD, A.E., DICKISON, W.C., MASSEY, J.R. & BELL, C.R. 1974. *Vascular plant systematics*. Harper & Row, New York / Evanston, San Francisco, London: 891 pp.

RAYNA-ROQUES, A. 1980. Les plantes aquatiques, étude et recolte. *In*: DURAND, J.R. & LEVEQUE, C. (Eds). *Flore et faune aquatiques de l'Afrique Sahelo-Soudanienne*. *I.D.T.* 44. ORSTOM, Paris: 69-73.

REICHELT, G. & WILMANNS, O. 1973. *Vegetationsgeographie*. Westermann, Braunschweig: 210 pp.

RICH, T., HACK, V. & MCMEECHAN, F. 2005. Vascular Plants. *In*: HILL, D., FASHAM, M., TUCKER., G., SHEWRY, M. & SHAW, P. (Eds). *Handbook of Biodiversity Methods. Survey, Evaluation and Monitoring*. Cambridge University Press, Cambridge: 305-321.

SAVILE, D.B.O. 1961. *Collection and care of botanical specimens*. Canada Department of Agriculture, Ottawa: 124 pp.

SCHEINER, S.M. 2003. Six types of species-area curves. *Global Ecology and Biogeography* 12: 441-447.

SCHEINER, S.M. 2004. A mélange of curves - further dialogue about species-area relationships. *Global Ecology and Biogeography* 13: 479-484.

SHMIDA, A. 1984. Whittaker's plant diversity sampling method. *Israel Journal of Botany* 33: 41-46.

SILVERTOWN, J. & CHARLESWORTH, D. 2001. *Introduction to Plant Population Biology*. Blackwell, Oxford: 224 pp.

SODERSTROM, T.R. & YOUNG, S.M 1983. A guide to collecting Bamboos. *Annals of the Missouri Botanical Garden* 70: 128-136.

SOULÉ, M.E. & KOHM, K.A. 1989. *Research Priorities for Conservation Biology*. Island Press, Washington DC: 97 pp.

STACE, C.A. 1989. *Plant Taxonomy and Biosystematics*. 2d ed. Edward Arnold, London: 264 pp.

STOHLGREN, T.J. 1994. Planning long-term vegetation studies at landscape scales. *In*: POWELL, T.M. & STEELE, J.H. (Eds). *Ecological Time Series*. Chapman & Hall, New York: 208-241.

STOHLGREN, T.J. 2006. *Measuring Plant Diversity. Lessons from the Field*. Oxford University Press, Oxford: 408 pp.

STOHLGREN, T.J., OWEN, A.J. & LEE, M. 2000. Monitoring shifts in plant diversity in response to climate change: a method for landscapes. *Biodiversity and Conservation* 9: 65-86.

STONE, B.C. 1983. Collecting Pandanaceae. *Annals of the Missouri Botanical Garden* 70: 137-145.

STRAIN, B.R. & BILLINGS, W.D. (Eds). 1974. *Vegetation and environment*. *Handbook of vegetation science* 6. Junk, The Hague: 193 pp.

TAYLOR, P. 1977. On the collecting and preparation of *Utricularia* specimens. *Flora Malesiana Bulletin* 30: 2831-2832.

TÜXEN, R. 1970. Bibliographie zum Problem des Minimum-Areals und der Art-Areal-Kurve. *Excerpta Botanica Section*, B 10: 291-314.

VOGEL, E.F. DE 1987. *Manual of herbarium taxonomy: theory and practice*. UNESCO Regional Office for Science and Technology for South East Asia (Indonesia) ROSTSEA, Jakarta: 164 pp.

WALTERS, D.R. & KEIL, D.J. 1996. *Vascular Plant Taxonomy*. 4th ed. Kendall/Hunt, Dubuque: 608 pp.

WHITTAKER, R.H. 1977. Evolution of species diversity on land communities. *Evolutionary Biology* 10: 1-67.

WILLI, Y. & FISCHER, M. 2005. Genetic rescue in interconnected populations of small and large size of the self-incompatible *Ranunculus reptans*. *Heredity* 95: 437-443.

WILSON, E.O. (Ed.). 1988. *Biodiversity*. National Academies Press, Washington DC: 538 pp.

WOMERSLEY, J.S. 1981. Plant collecting and herbarium development. *FAO Plant productions and protection papers* 33: 1-137.

5. Acknowledgements

We thank Ruth Eastwood, Kew, for comments and correcting our English.

6. Appendix - Collection (passport) form

documentation of the field work

collection date ..... collection site number .....

name(s) of field worker(s) .....

institution .....

taxon data

taxon name or preliminary taxon name .....

vernacular name, language .....

herbarium voucher number ..... photos .....

colour of flower .....

additional notes (*e.g.* life-form, habit, size, type of underground organs, scent) .....

.....

phenological status: more flowers than fruits / more fruits than flowers / only fruits / fruits already dispersed

frequence: rare / few / frequent / very frequent / highly frequent *(tick)*

population and ecological notes

habitat .....

vegetation cover .....

canopy cover ..... % of bare ground .....

vegetation notes .....

associated species .....

..... EUNIS habitat code .....

human use .....

soil .....

geographical notes

country ..... region .....

location ..... coordinates .....

altitude ..... map datum .....

slope: level 0-5 % / undulating 6-10% / rolling 11-20% / moderate 21-30% / steep >30% (*tick*)

source of coordinates: topographic map / GPS / Google Earth (*tick one*)

population and site notes, circumstances of the field work (*e.g.* population size, fitness, observations) .....

regional administrations, scientists and florists .....

collection permission

used literature (national / regional flora, determination keys)

Chapter 15

Sampling insects: general techniques, strategies and remarks

by

Patrick Grootaert
Royal Belgian Institute of Natural Sciences (RBINS)
Rue Vautier 29, B-1000 Brussels, Belgium
Email: pgrootaert@yahoo.co.uk

Marc Pollet
Research Institute for Nature and Forest (INBO)
Kliniekstraat 25, B-1070 Brussels, Belgium
Email: marc.pollet@inbo.be

Wouter Dekoninck
Royal Belgian Institute of Natural Sciences (RBINS)
Rue Vautier 29, B-1000 Brussels, Belgium
Email: wdekoninck@natuurwetenschappen.be

Cees van Achterberg
National Museum of Natural History Naturalis
P.O. Box 9517, NL-2300 RA Leiden, The Netherlands
Email: achterberg@naturalis.nnm.nl

Abstract

Sampling insects requires knowledge of their biology, preferred habitats and activity patterns. An overview is given of the most frequently applied collecting and recording techniques and the insect taxa that they gather in largest numbers. Sampling strategies can be deduced for each of the included taxonomic groups. Following techniques are described and recommendations and restrictions are given for them: 1. Active collecting: pooter, portable suction devices, sweepnet, visual observation; 2. Passive collecting: coloured pan traps, emergence traps, sticky traps and suction traps. For light traps, Malaise traps and pitfall traps we refer to other chapters.

Keywords: Sampling strategies, coloured pan traps, suction traps, emergence traps, sticky traps

1. Introduction

It is virtually impossible to attempt at collecting all species of one particular taxonomic group with only one sampling technique. And it is considered very unlikely to collect all of them even with several methods. This is not only due to the specific life histories of the different species, and their numbers in the field, but also to features of the recording methods used. Preferably, at least two or three collecting techniques, and visual observations in the field are mandatory to get a representative idea of the present species richness. In a canopy sampling campaign for weevils (Coleoptera: Curculionidae), the three methods applied (fogging, sticky traps, light traps) each yielded a very large number of species, but proved strongly complementary in terms of collected species (Missa, 2000) (Fig. 1).

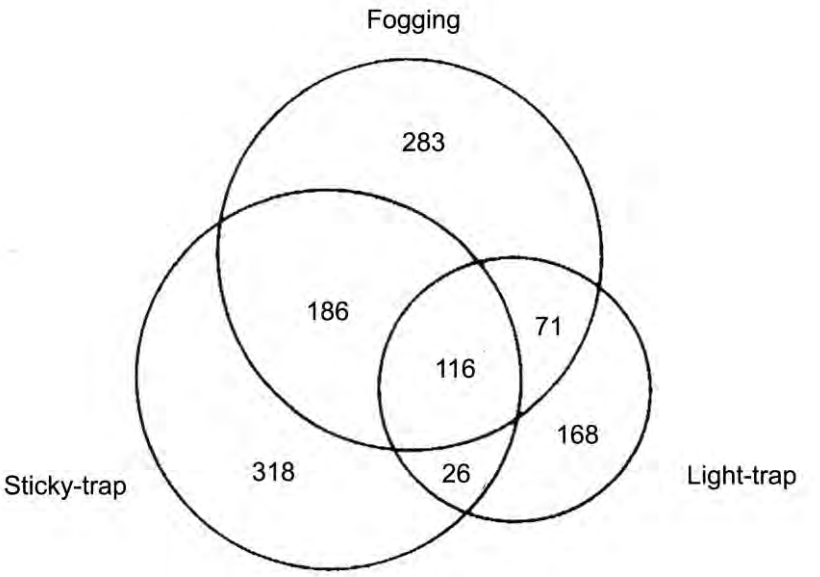


Fig. 1. Weevil species richness (Coleoptera: Curculionidae) as established by three collecting techniques in lowland rainforest in Papua New Guinea (Missa, unpublished data).

Sampling insects requires knowledge of their biology, preferred habitats and activity patterns. Like most invertebrates, many insects show oscillating population densities with cycles from 3 up to 10 years (Hunter & Price, 1998) (Fig. 2). In low density years, species' populations are difficult to measure and might give the impression that the habitat represents suboptimal conditions. In temperate and tropical climates, insects show a specific annual activity pattern, often referred to as phenology (Tauber & Tauber, 1981). In temperate regions these patterns are triggered by photoperiod in combination with temperature and humidity (van Asch & Visser, 2007), which renders species being most active during spring, summer, autumn, and even winter. Some species even have several generations per year disjunct in time. Apart from monsoon conditions it

remains unclear what exactly triggers phenology in the tropics, certainly around the equator, where photoperiod and temperature are subequal throughout the year. In tropical forests the fruiting of trees may be one of the triggers.

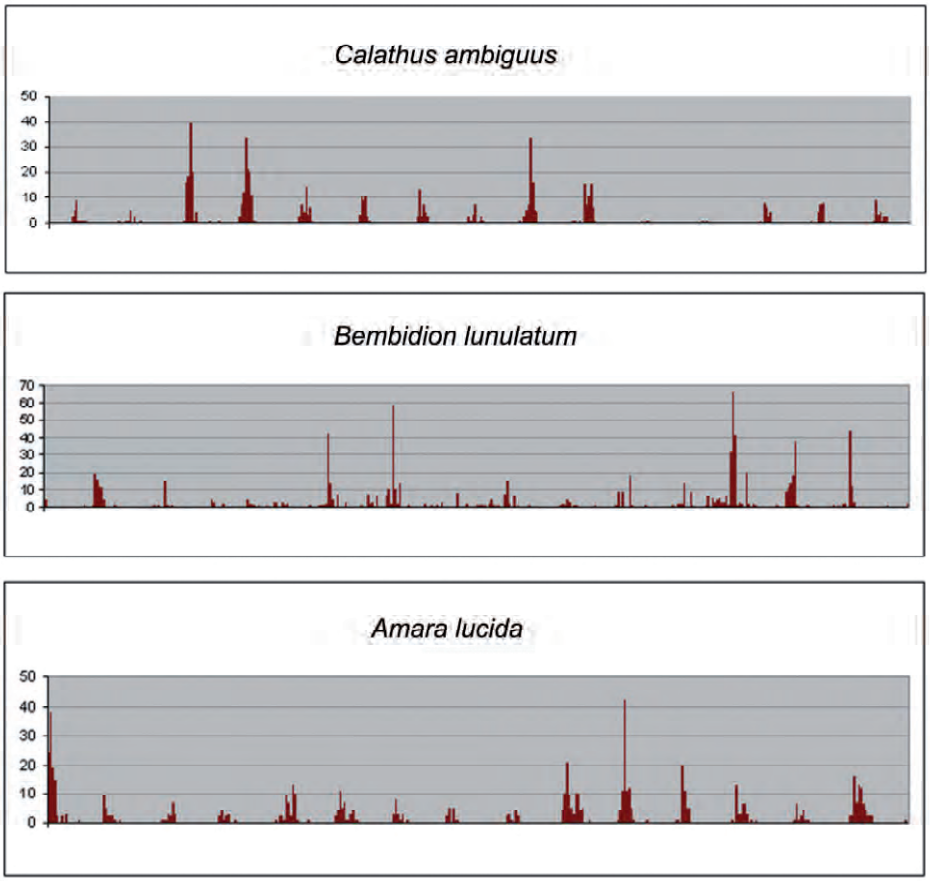


Fig. 2. Cycles of annual variations in population density of three ground beetle species (Coleoptera: Carabidae) resulting from pitfall traps over 15 years: the periodicity of population peaks varies between 5 years in *Calathus ambiguus*, and 10 years in *Amara lucida*. In low density years, populations are hard to establish (Desender, unpublished data).

An All Taxa Biodiversity Inventory (ATBI) *sensu stricto* is an illusion as well. It is not feasible to record all species at one particular site, even when sampling continuously, year-round and using different techniques. But the strategic employment of a particular combination of trapping techniques might yield a sufficiently representative portion of the species richness. Each collecting device has been constructed to gather particular taxa as efficient as possible, using species' features as mobility and attraction: *e.g.* Malaise traps collect a very diverse fauna of mainly diurnal flying insects; pitfall traps focus primarily on soil-

dwelling invertebrates, whereas coloured pan traps and light traps attract flying insects during the day and night respectively (Missa *et al*., 2009).

Before initiating a sampling campaign, the goal of the action should be very clear. Also, aspects as coverage and intensity of the sampling in time and space, practical issues, treatment of material before preparation and logistics, and the handling of possible by-catches or residue samples should be taken into account prior to the start of the campaign.

It is very important to choose the collecting method and devices according to preservational aspects. Many taxa are to be dry-mounted by pinning or gluing onto paper cards as a standard preservation method. Collecting devices using fluid fixation agents prevent satisfying results in many cases (as for all Lepidoptera, pilose and coated specimens), and require ultimate liquid specimen preservation, also dependent on fixation agent, collecting periods, temperature, etc. In these cases passive collecting devices can be used without fixation fluids, but have to be serviced in short intervals. So fixation and preservation fluids must be selected according to the final purpose of the gathered specimens (*e.g*. DNA extraction requires 100% ethanol). See chapter 18 by Krogmann & Holstein.

Traps have been designed for each stratum, from the soil surface level (to collect soil-dwelling and weak flyers), over the near-soil stratum (most of the flying insects in herb and lower canopy levels) up to the upper canopy. The canopy can hold an unprecedented biodiversity as shown by Erwin (1982) who observed that about 2/3 of the arthropods of a dry tropical forest occur in the canopy. The present chapter deals only with the near-soil stratum. Collecting strategies and techniques for soil-stratum and canopy invertebrates are treated in chapters 9 and 8, respectively.

A clear difference should be made between discontinuous or occasional, and continuous sampling techniques, and both have their advantages and shortcomings. If practically possible, continuous sampling with traps is recommended because of the relatively low service time (especially as compared to the time needed to collect the same species richness actively), and the fact that traps remain in operation regardless of weather conditions.

Trapping devices can also be separated into attraction and interception traps. Attraction traps employ the phenomenon of attraction of the species by the trap, generated by agents such as light, colour, odour and others. Interceptions traps, on the contrary, form an obstruction on the path of organisms and lead them to a collecting device. A number of traps combine both sampling methodologies.

A third way to divide sampling activities is based on the involvement of the collector himself during the collecting activity and in this frame, active and passive collecting are distinguished. The former approach implies the direct and active involvement of the collector who effectively moves (around) in search for the focal taxa. Active sampling encompasses visual observation, sweep netting and the use of pooters and related recipients. Passive collecting, on the other hand, is based on the movement of the focal taxa towards the trapping device. This methodology includes all kinds of continuous traps such as Malaise traps, pan and pitfall traps, fixed suction traps, sticky traps, light traps and emergence

traps. All of these collecting techniques are presented below, except for Malaise, light and pitfall traps, which are dealt with in chapters 17, 16 and 9, respectively.

Collecting / recording techniques relevant for ATBIs of insects

Table 1 presents an overview of the most frequently applied collecting / recording techniques and the insect taxa that they gather in largest numbers. From this table, recommended sampling strategies can be deduced for each of the included taxonomic groups. Hereunder, the different techniques are described, and recommendations and restrictions are given.

Collecting techniques (see Text)	Active collecting					Passive collecting						
	2.1 pooter	2.2 portable suction devices	2.3 sweepnet	2.4 visual observations	*fogging*	3.1 coloured pan traps #	3.2 emergence traps	3.3 light traps	3.4 Malaise traps	3.5 sticky trap	3.6 suction traps	*pitfall traps*
Collembola	□	■			□				□	□		■
Thysanura					□				□	□		■
Ephemeroptera			■					□	□			
Plecoptera			■					□	■			
Blattodea			□		■	■		□	□	□		□
Isoptera					□			■	■			
Orthoptera			■		□			□	□			
- Tettigonoidea			□	■	□			□	□			
- Acridoidea			□	■								□
- Tetrigidae			□		□			□	■			□
Embioptera					□			□	■			
Psocoptera			□		□				■			
Hemiptera			□						■			
- Cicadomorpha			□		□			■	□			
Thysanoptera			■		□				□			
Neuroptera			□		□			□	■			
Coleoptera												
- xylobionts (*e.g.* Cerambycidae, Scolytidae)			□		■		■	■	□	□		
- ground-dwelling beetles (*e.g.* Carabidae)								□				■
- phytophagous beetles (*e.g.* Chrysomelidae)			■		□			□				
- aquatic beetles (*e.g.* Dytiscidae)			■					□				

Strepsiptera							■		
Diptera		■		■ $^{W, Y}$	□		■		
- apterous/brachyterous flies	□				□				■
- Dolichopodidae		■		■ $^{W, Y}$	□		■	□	
- remaining Empidoidea		■		■ $^{W, Y}$			■		
- Phoridae				■			■		□
- Syrphidae		■		□			■		
- Stratiomyidae				□			■		
- Tabanidae							■		
Mecoptera		■			□	□	□		
Lepidoptera									
- Rhopalocera		■		□ ^{W}			□		
- Heterocera						■	■		
Hymenoptera		■		□			■		
- Apoidea		□		■ ^{Y}			■		
- Cynipoidea		□	□		□		■		
- Parasitoids		□	□	□ $^{W, Y}$	□	□	■		
- Formicidae		□	□	□ $^{W, Y}$		□	■	□	□
- Ichneumonoidea		□		□ $^{W, Y}$			■		
- Pompilidae		□		□ $^{W, Y}$		○	■		
- Symphyta		□		□ $^{W, Y}$			■		
- Vespoidea		□		□ $^{W, Y}$		○	■		

W: white; Y: yellow pan traps

Table 1. Overview of techniques used to collect insect orders and some selected superfamilies and families. Only taxonomic groups for which at least one technique can be assigned as recommended are included. Explanation of covered collecting techniques follows the structure of the chapter; techniques not treated here are indicated in italics. Most recommended techniques are indicated as ■ (if two or more techniques are in this category, they are considered as equally recommended); useful, supplementary techniques indicated as □. If no techniques are indicated for a certain order, recommended techniques for the underlying families differ greatly.

2. Active collecting

2.1. The pooter

A pooter consists of a collecting jar closed by a cork or polymer stop with two flexible tubes inserted into it, a mouthpiece tube to aspire and a collecting tube to suck up the insect. At the inner end (in the collecting jar), the mouthpiece tube is covered by a fine gauze (Fig. 3) to avoid insects from entering the collector's mouth. Small insects are collected by positioning the collecting tube on top of the insect and abruptly sucking it up into the collecting jar. In between collecting actions, the outer end of the collecting tube must be covered or blocked by a stop to avoid the insects from escaping. Finally, the insects can be transferred to a killing jar or preservative by gently removing the stop. This method is widely used

to collect insects from all kind of surfaces (rocks, fences, tree trunks, etc.), from crevices and even from sweep net samples. This method is particularly interesting to gather insects that tend to stick to these substrates, and thus cannot easily be collected with a sweep net.

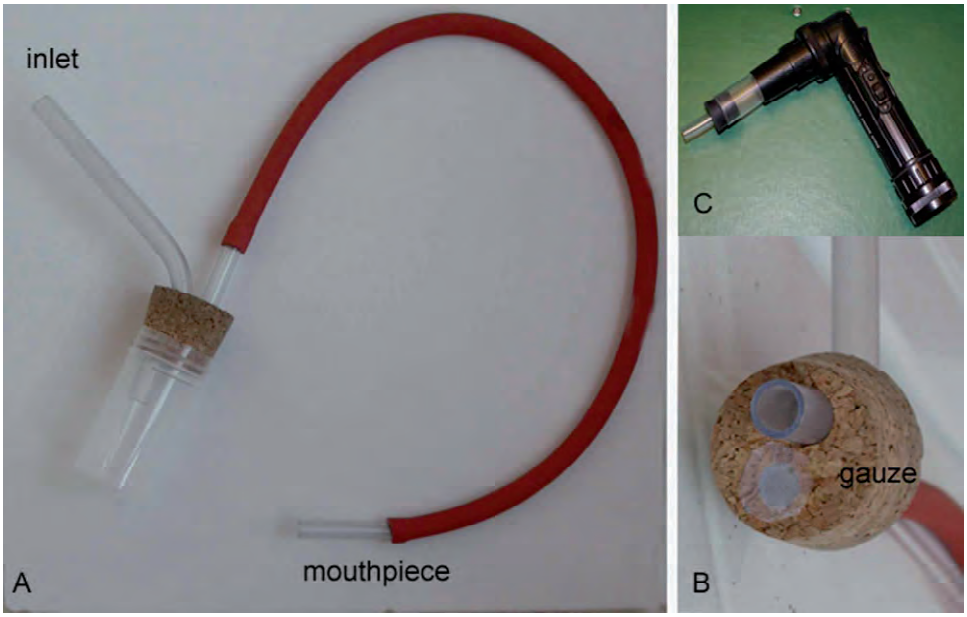


Fig. 3. A. Mouth-pooter; B. Gauze at the inner end of the mouthpiece tube prevents insects from being inhaled; C. Electric pooter (Photos A & B by Patrick Grootaert; C on http://svalbardinsects.net/index.php?id=64) .

Recommendations:

- Use a distinctive mouthpiece tube to avoid confusion with the collecting tube;
- Glass collecting jars are prone to get broken, so transparent plastic vials are safer. However, be aware that some types of polymer corrode when in contact with a killing agent;
- Transfer the collected insects regularly to the killing jar so that the pooter jar does not become too crowded. By putting a piece of paper tissue in the pooter jar, the time interval between collecting actions can be increased and collected insects do not get too damaged during the trip;
- To kill the collected insects, a piece of paper tissue with some volatile killing agent can be deposited into the pooter jar prior to their transfer into a larger killing jar. Take care that the killing agent is entirely evaporated before the pooter is used again.

Restrictions:

- While aspiring, enormous amounts of germs (fungi, bacteria, viruses, mites and their eggs, springtails, etc.) can be inhaled which might cause damage to the respiratory system. It is also highly recommended when collecting insects from excremental surfaces to use a rubber bellow on the mouthpiece tube instead or an electric pooter;
- Ants and certain beetles emit noxious products when disturbed, and in these cases an electric pooter is recommended.

In the case of tree-trunk dwelling flies, an alternative and safer method consists of a transparent vial (a recipient with a diameter of 3 cm and a depth of 7 cm is very practical) that is rinsed with some alcohol solution. This leaves a thin wet layer on the inside of the vial in which flies and other flying insects get entangled while flying up when the vial is quickly put on top of them. In this way, a surprisingly high number of specimens can be collected during one collecting action before being transferred to an alcohol solution. This method is superior to all others for collecting *Medetera* spp. (Diptera: Dolichopodidae) and other arboreal trunk-dwelling long-legged flies. This method is well suitable for specimens that are ultimately wet preserved, but only to some extent to collect dry preserved insects.

2.2. Portable suction devices

D-VAC is a portable aspirator activated by a gasoline engine and carried on the back of a person. The advantage of D-VAC vacuum sampling as compared to other sampling techniques is the more complete extraction of tiny invertebrate species, and immature forms of even larger species from the environment. Due to the pressure built up by conventional nets while sweeping, insects of low body mass simply do not enter them as they are caught in the overflow of air pressure built up as the net is sent through the air. By applying suction to the collecting bag, this inertia of air at the entrance of the net is overcome and tiny forms are collected more readily. Using a similar motion as is done while swinging an insect net, the D-VAC is also suitable to sample more heavy insects like caterpillars, beetles, etc. For fragile insects like many flies, sweep netting is preferred over suction trapping, although the latter method might be applied successfully to capture cryptic species that occur in dense vegetations, within tussocks and in *e.g.* rot-holes of trees.

2.3. The sweep net

Sweep nets come in all shapes and sizes, each designed for a particular insect group (Stubbs & Chandler, 1978). Both the net shape and sweeping technique affect the yield as commented upon by Chalcidoidea (Hymenoptera) specialists (Anonymous, 2004). While employing a sweep net, the collector not necessarily targets a specific specimen, but sometimes carries out a random sampling of the fauna present in the vegetation or on the soil surface. The species diversity in

sweep net samples often resembles that of Malaise trap yields (Guevara & Aviles, 2009).

The sweep net is by far the most widely used device to collect insects, and has been the most important one for the past centuries. Its success can be explained by its practical use and the fact that it can be employed in almost every possible habitat, except for densely vegetated or inaccessible sites (reed marshes, mangroves, etc.) and thorny vegetations. Moreover, it is ideal for short-term large scale inventories as the gathering of the separate samples is not time-consuming and several sites can be visited during the same day. Also, it does not require the collector to return to the same site more than once to collect the yields.

When using a big-sized net selected insects can be gathered with a pooter. This holds true for small specimens only and is not feasible for *e.g.* Lepidoptera and medium-sized to large arthropods. If the entire content is to be conserved, the yield is gathered in the tip of the net by sweeping the net a few times and closing it manually. If the specimens must be stored dry, the tip can be put in a jar with a knockdown agent like ethyl acetate to kill the specimens. Subsequently, the sample can be exposed on a white sheet for immediate sorting. The collector should make sure that the specimens are dead (caterpillars and beetles might be harder to kill in this way). If the specimens are stored wet, then the tip of the net with the yield can easily be emptied in a collecting jar with an alcohol solution.

Beating vegetation with a strong sweep net or with a stick and subsequently collecting the fallen insects on a sheet or in an umbrella is an alternative way to collect arthropods like spiders, beetles, bugs and caterpillars. However it is not highly recommended to maltreat vegetation in a nature reserve, especially in the presence of park guards.

***Recommendations*:**

- Use a net with the right mesh size; dipterists require a finer mesh size than *e.g.* butterfly or dragonfly collectors. Sweeping nets for sweeping through thorny vegetation must be made of a stronger fabric (*e.g*. linen), at least around the clamp to avoid ruptures;
- Transfer the sample to a collecting jar after a limited number of sweeps, depending on the size of the sample (this requires some experience). Samples collected during a long sweeping session tend to contain a high ratio of damaged specimens;
- Sweep gently (over) the vegetation; insects will fly up, end up in the net and will not be damaged, nor will the vegetation. If sweeping too severely, leaves and branches will end up in the net, damaging the specimens;
- Use an eversible stick which makes the collecting radius substantial larger;
- Take care when manipulating the sample (in the tip of the net) and watch out for stinging insects, especially when you are allergic;

- Join an experienced entomologist on one of his trips; you will learn more and much faster than studying manuals. Every entomologist has his personal technique that affects the yields.

***Restrictions*:**

- Sweep netting of vegetation cannot be done when vegetation is humid or highly thorny. Fragile insects will be severely damaged which renders them useless for identification. As insect activity only starts when the temperature is sufficiently high, collecting with sweep nets becomes only efficient when the collecting sites are exposed to the sun. In practice, collecting starts best not before 8:00 a.m., especially in strongly wooded habitats, and lasts until the late afternoon (when the weather is dry). Poorly vegetated sites like beaches, especially in the tropics, are best avoided at noon when insects escape from the soaring temperatures and hide in the soil or on the soil surface within dense vegetations.

2.4. Visual observation

Visual observation is a technique that should not be underestimated. Moreover, it is the innate feeling of most entomologists nowadays that they spend too little time in the field to learn about the whereabouts of their animals of interest. Instead, sampling is mostly done as efficient as possible, using all kinds of trapping devices which can yield very large amounts of species and specimens but only rarely uncover aspects of their life history (see further). Observing insects in their natural habitat yields information on their behaviour, commotion and preferred (micro)habitats. *E.g.* many long-legged fly species (Diptera: Dolichopodidae) in the tropics demonstrate very specific habitat affinities and are sometimes entirely confined to *e.g.* springs, waterfalls, rapids and even splash zones of rocks amid rivers.

Well-sized specimens can be collected by hand or with a jar or vial, respectively. In this way, non-flying arthropods from substrates and from under rocks, stones or bark are usually collected.

During visual observation, specimens can be photographed and pictures and related information can be stored using PDAs (personal digital apparatus), which have rather recently been developed. Recording using only visual observation is only suitable for taxonomic groups that are easily recognized in the field. In all other cases, it is strongly recommended to collect voucher specimens for confirmation of their identity in the laboratory.

3. Passive collecting

3.1. Coloured pan traps

Next to sweep nets and Malaise traps (see chapter 17), the most frequently employed technique to collect flying insects is undoubtedly pan traps. These traps were initially used in pest species sampling, but more recently have

become part of the standard biodiversity assessment instruments mainly applied by North American entomologists, but only few European researchers (Baillot & Tréhen, 1974; Pollet & Grootaert, 1987, 1991, 1996). In contrast to Malaise traps and sweep nets that are manufactured exclusively for the collecting purpose, any kind of device that holds a certain amount of (preserving) liquid and that features a colour attractive to the focal taxon is suitable as pan trap. The material can range from garbage bags, vinyl sheets, plastic food trays to aluminium roasting pans, but the most practical are definitely round plastic bowls that are weather-proof (the colour should not change over time). The specific type to be used largely depends on sampling site attributes (*e.g.* accessibility, distance to the collector's residence). Nearby sites can be sampled with large and heavy pan traps (see Pollet & Grootaert, 1987, 1991), but most recommendable in all situations are light-weight and easily stackable types such as 12 oz plastic partyware bowls (see http://www.partypro.com). These bowls that come in 41 different colours have a flat rim of 2.3 cm, an inner diameter of 15.4 cm and a depth of 3.7 cm. They were recently employed successfully during an expedition in Ecuador (Pollet, unpubl. data) (Fig. 4A, B). Unfortunately, these devices do not seem to be found easily in Europe.

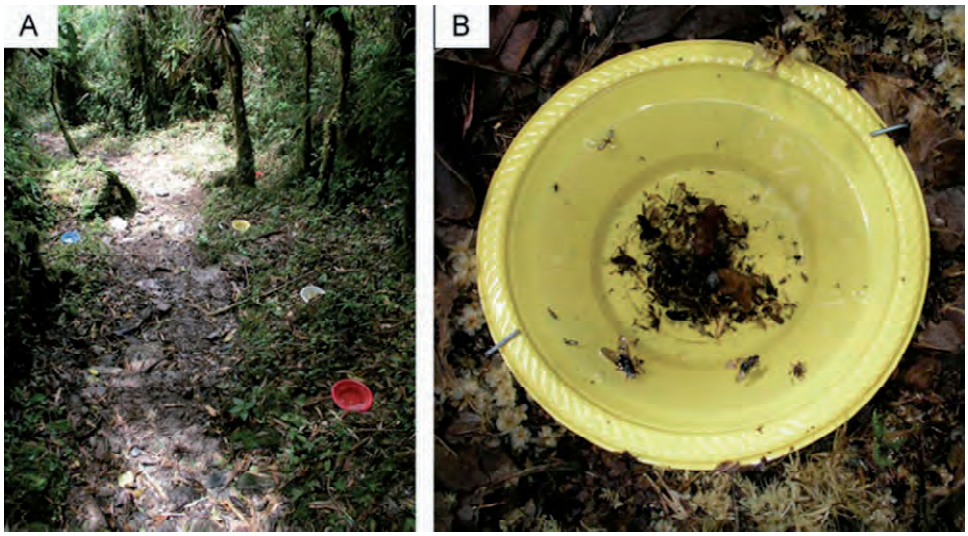


Fig. 4. A. Different coloured pan traps along a trail in a forest in Ecuador; B. detail of the insects trapped in a pan trap. (Photos by Marc Pollet).

One of the most significant advantages of the use of pan traps is their versatility: not only can the size and shape be varied infinitely but also the trap colour and its installation can be adapted greatly in order to optimise the sampling process (see Pollet & Grootaert, 1994). Traps with a bright yellow colour (often referred to as Moericke's traps) are by far the most widely used and attract a broad spectrum of low-flying insects, in particular Hymenoptera and predacious flies. Also white pan traps repeatedly proved to be excellent devices to collect certain fly families *e.g.* Syrphidae and Dolichopodidae: one trap type with a diameter and depth of approx. 9 cm yielded on average 116 and 248 dolichopodid specimens during one season in reedmarsh (Pollet, 1992) and marshland sites, respectively

(Pollet, 2001). These sampling campaigns gathered a total of 73 and 68 species using 77 and 54 traps, respectively. Moreover, a comparative study by Pollet & Grootaert (1994) involving white, yellow, and bluish green pan traps revealed that white and yellow traps collected a comparable number of species; the higher number of specimens yielded by yellow traps was explained by only one very abundant species. Most dolichopodid species thus appear to be most attracted by yellow and white and less by other colours as blue and red. This, however, does not hold true for arboreal dolichopodid species (*e.g. Medetera* spp., *Neurigona* spp., *Sciapus* spp.) that are collected in highest numbers in blue, and soil-dwelling species (*e.g. Campsicnemus* spp.) that are most numerous in red (and blue) pan traps (Pollet & Grootaert, 1987). Actually, thus far *Australachalcus melanotrichus* Pollet & Stark, a species that breeds exclusively in rot-holes of trees, has only been gathered by blue or bluish green traps in multicolour pan trap campaigns (Pollet, unpubl. data). Also other dipteran families with larvae that breed in plant tissue such as leaf miners (Chloropidae) and fruit flies (Tephritidae) are most attracted by blue pan traps.

The installation height also has a substantial impact on the yields. In general, pan traps sunk into the soil are most productive, both in terms of species and specimens (Pollet & Grootaert, 1987, 1991). Again, some species like the xerophilous *Chrysotus gramineus* (Fallen, 1823) and arboreal species are collected more abundantly in traps at 60 cm height (Pollet & Grootaert, 1987), or traps level with vegetation height (Pollet, 2001). As a result, blue or bluish green traps installed at a certain height are best employed if the research focuses on arboreal species communities. If a short-term assessment of the overall species diversity is the main aim, yellow or white pan traps are preferably used. And in case of faunas with a largely unknown ecology, a combination of yellow, white, red and blue coloured traps can be strongly recommended (as the distribution of species of the differently coloured traps holds information on their ecology).

Pan traps thus can be used in every terrestrial and semi-aquatic habitat but are most commonly installed at soil surface level. Traps that are installed on the soil only yield a fraction of the soil-dwelling fauna of *e.g.* carabid beetles and spiders, which are abundantly trapped in pan traps dug into the soil with their rim at soil surface level. In either case, they should be fixed to the soil by metal pins or any other device that prevents displacement. Pan traps can be put simply on the soil in habitats with a well developed herb layer, or sites that are subject to regular but mild flooding. In drier habitats traps are better sunk into the soil and are preferably deeper to prevent them from drying out.

Pan traps are usually filled for ¾ with water. A sufficient amount of detergent must always be added as a surfactant to break the surface tension. Depending on the servicing periodicity, salt can be added as a preservative. If traps are emptied daily or every two days, salt is not necessary, but it becomes absolutely essential with longer servicing intervals. A possible alternative that allows even longer sampling intervals is formalin solution. With a 5% solution as preservative, traps can remain in operation for at least 7 days, and for a fortnight with a 10% solution. Precipitation (rainfall) should be taken into account, especially in the tropics, which can cause a very quick and strong dilution. Deeper traps (over 5 cm) might reduce this effect, but are no guarantee for a good preservation of the

trapped specimens in the rainy season. To avoid the loss of (floating) specimens due to heavy rainfall, minute holes just below the upper rim of the pan trap work well as drainage. Further on, especially in forests and wooded habitats in general, falling leaves or branches might cover the traps largely to entirely, blocking any insect to be trapped. This can be prevented by constructing a framework of thin branches or metal wire covering the trap. As this can be rather time-consuming, it is more practical to service the traps at sampling intervals of at most 5 to 7 days.

The servicing process starts with removing large objects such as leaves, twigs, and vertebrates that might obstruct the collection of the trapped invertebrates and accelerate their decomposition. The remaining contents are subsequently scooped out with a fine mesh aquarium net while collecting the preservative liquid (in a supplementary trap) for reuse (after addition of some fresh solution if necessary). In order to recover the entire content, the net might need to be dragged several times gently near the bottom in one direction. The content of one trap can be kept separately or be pooled with the contents of other traps, depending on the specific objective of the sampling campaign. If the preservative liquid is significantly coloured (mostly by leaves), fresh solution should be used. The contents are transferred to collecting jars or (better) self-sealing plastic bags (*i.e.* whirl-pack type) and properly labelled. Preferably a 90% ethanol solution is added as preservative.

The pan trap technique holds a number of advantages as compared to Malaise traps (Pollet, 1988): (i) they are less striking in the field and as such less subject to damage or removal; (ii) yields are usually fair but not as massive as those of Malaise traps which allows processing in proper time; (iii) consequently, per sampling site a number of traps (Fig. 4A) can be installed to gather information on the heterogeneity of the fauna without jeopardizing the processing of the samples; and (iv) information on the ecology can be gathered using traps of different colours. Nevertheless, it is strongly recommended to employ both techniques in combination as they are largely complementary: a preliminary analysis of samples from Braulio Carillo National Park (Costa Rica) revealed that both trap types collected an identical number of species (26), but shared only 30% or 12 of the total number of species collected (n = 40) (Pollet, 2002). Actually, comparing the yields of both trap types also provides information on the flying activity and frequency of the trapped species.

3.2. Emergence traps

Emergence traps are based on the phenomenon that most insects move up towards the light after emerging. These traps very often reveal species that are rarely collected with other trapping techniques. This was recently illustrated by a field experiment (Fig. 5) along the Belgian coast (see further) that yielded 16 species of Diptera. Two of the species proved new to the Belgian fauna which was surprising as the same beach habitats have been sampled intensively for the past 30 years (Grootaert *et al*., in litt.). Moreover, this kind of collecting method also gathers information on larval development time and food preference.

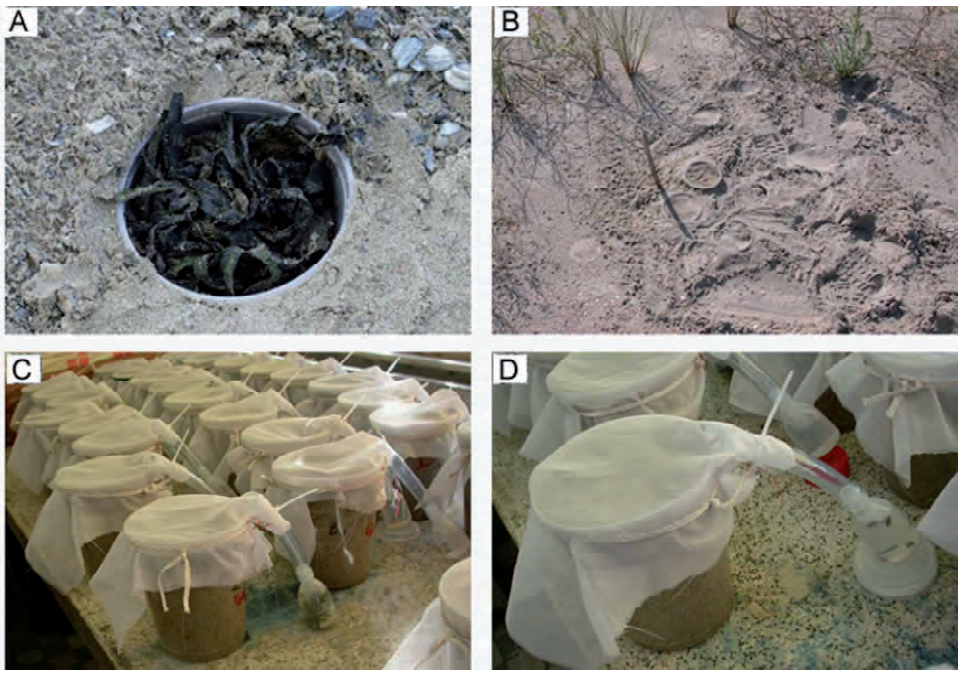


Fig. 5. Collecting insects on the beach with baited emergence traps. A. Freshly cut seaweed is put on top of a vial that is filled with sand and B. dug into the beach for two weeks; C. Subsequently the vials are transferred to the laboratory and D. a cover and collecting jar filled with 70 or 90% alcohol is attached. Emerging insects are collected weekly during a period of two months. (Photos by Wouter Dekoninck & Patrick Grootaert).

Several types of emergence traps are currently available. Some are installed for some period of time in the field, where emerging insects are gathered. Other types (see above) are baited to attract insects that deposit eggs into the intentionally provided substrate, and are returned to the lab for the larvae to accomplish their development and the adults to emerge.

Emergence traps in the field

A first type of emergence trap usually consists of a large pyramidal structure made of black fabric (nylon or other tissue) with a collecting jar on top (Fig. 6). Commercial wasp traps can be used as collecting jar and filled with alcohol. It is still unclear to what extent the climatic conditions within this trap are affected and what fraction of the present fauna eventually ends up in the collecting jar (Glen, 1976).

To collect xylobiont arthropods in the field on standing dead wood, an emergence trap can be attached to, or even constructed around the tree (Fig. 7).

Fig. 6. Emergence trap in the field. The collecting jar is a plastic commercial wasp trap filled with 70% alcohol. (Photo by Wouter Dekoninck).

Fig. 7. Emergence traps fixed around a dead tree to collect emerging xylobiont insects. (Photo by Kris Vandekerkhove).

Emergence traps in the laboratory (see also Berlese and Winkler samples, chapter 9)

Adult insects, especially Diptera, that are not easily collected with the usual sampling techniques are sometimes obtained by gathering soil, litter, dung, mushrooms, decaying fruits, wood or debris in the field, and transferring it to the laboratory for (adult) insects to emerge. Soil samples should remain undisturbed. Dead branches can be placed in large containers and can even be left for months or years as the developmental time of some xylobiont species last

several years. Xylobiont (beetle) species generally emerge in spring (April until June in northern temperate regions) and in this period, traps should be checked regularly.

- In some cases, insects are attracted by bait in order to deposit eggs. The substrates holding the eggs and larvae are subsequently transferred to the lab for the adult insects to emerge. This methodology was recently applied along the Belgian coast: jars filled with sterile beach sand were baited with freshly cut seaweed, and left in the field for about two weeks. It was assumed that fly species inhabiting the littoral zone would be attracted by the bait and deposit their eggs in the plant material. Minute holes in the bottom of the jars were provided for drainage to prevent the developing larvae from drowning. After two weeks, the jars with the soil and plant bait were brought into the lab where they were covered with a lid and a collecting jar was attached.
- A similar method is often used to collect parasitic species (mainly wasps and flies), by actively collecting the hosts in the field and rearing them in the lab. This approach enabled Dan Janzen to build an accurate idea of the tachinid parasite fauna (Diptera: Tachinidae) of caterpillars in the Santa Rosa National Park (Costa Rica) (Smith *et al*., 2006; see also Stireman *et al*., 2009).
- In each type of emergence trap, special attention should be drawn to the orientation and position of the collecting jar. As many emerging adult insects tend to be attracted by light, the jar opening is preferably on top of the trap and has a colour that is substantially lighter than the rest of the trap (Fig. 8). The collecting jar is best filled with an alcohol solution.

Recommendations:

- This method allows the collector to gather information on generation time and diet of the investigated species;
- Emergence traps in the laboratory are preferably held at room temperature (approximately 18-20°C);
- The humidity of the samples in the laboratory should be checked regularly. Samples that are too humid will cause mould and will stimulate mites to develop. An appropriate aeration is recommended in this case. Samples that dry too fast will cause a stop in the development of the insects or their death. If laboratory temperatures might be rather high (*e.g.* in summer), keeping the samples moist might be useful.

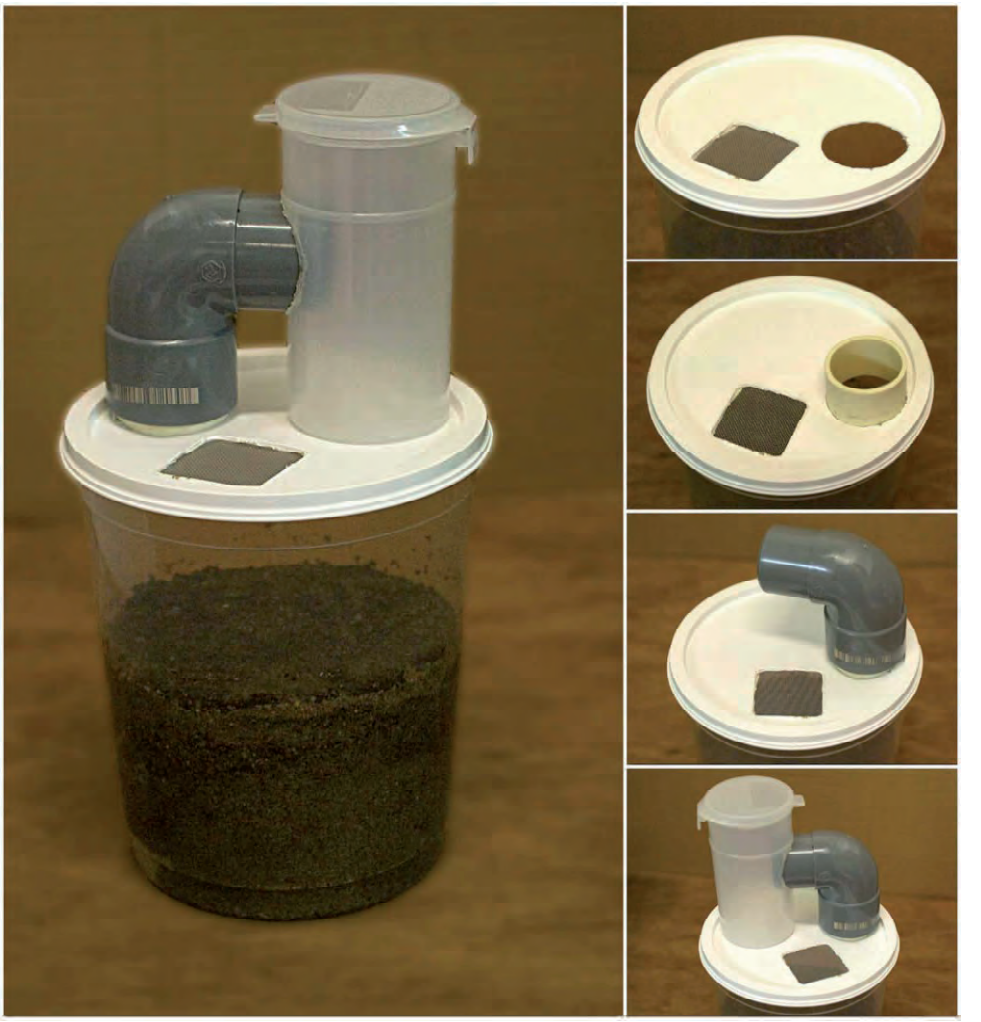


Fig. 8. Collecting jar of an emergence trap made of plumbing tubes. No glue is needed to fix the separate parts except for the mesh. (Photos by Filip De Block).

3.3. Light traps

Light traps are operated at night and are most effective from sunset till after midnight with clouded skies. Especially drizzly weather conditions are very productive, both in terms of species and specimens. This technique is generally applied for the collection of moths, scarabaeid beetles (Coleoptera, Scarabaeidae), and some Hemiptera and Hymenoptera. This trapping method is dealt with in chapter16.

3.4. Malaise traps

Next to the sweep net, Malaise traps are the most widely employed insect collecting devices since the 70'ies. They work unselective and often yield high

insect diversities with huge amounts of specimens. Sufficient time should be reserved for timely processing of these large samples. This collecting method is dealt with in detail in chapter 17.

3.5. Sticky traps

Sticky traps constitute of coloured sheets covered with a thin layer of weather-proof glue. They are made of waxed cardboard, glass, wood, plastic cups, plastic sheets or trap boards, empty milk cartons, red apple spheres or any other surfaces. The sheet's colour represents the attractive agent and depending on the applied colours, particular insect groups will be trapped. Glue types that are applied to this kind of traps are transparent. Attractants can be applied in combination with the glue to lure flying or crawling insects. Tanglefoot Tangle-Trap insect trap coating is often used as adhesive and remains sticky during the entire collecting period (Fig. 9).

Fig. 9. Sticky traps: glue-covered white wooden boards are pulled up 20-30 m high in the canopy of rain forest in Papua New Guinea in order to observe dispersal of weevils (Coleoptera: Curculionidae) between trees. (Photo by Patrick Grootaert).

***Recommendations*:**

- Unlike other traps, sticky traps can operate in inaccessible places such as the upper canopy (including tree trunks), and on top of water surfaces;
- Due to their versatility, sticky traps of different sizes and colours can be produced depending on the specific collecting purpose, similar to pan traps (see 3.1).

***Restrictions*:**

- Insects collected with sticky traps are very hard to detach without causing damage or the loss of body parts. The technique is therefore mainly used for the collection of large insects such as beetles and wasps. The glue is usually dissolved with kerosene, which is highly inflammable;
- Another type of sticky trap consists of a transparent plastic sheet with glue on both sides and attached to tree trunks. This technique should not be employed in areas with rich and endangered arboreal lizard or amphibian faunas.

3.6. Suction traps

Different kinds of suction traps are currently available: traps of the Rothampsted type are high towers that suck in air at a height of at least 10 m, and are mainly used for the monitoring of pest species like aphids (Hemiptera: Aphididae) or gnats (Diptera: Ceratopogonidae). As such, they do not seem particularly fit for ATBI purposes.

Suction traps can also be combined with attractants. The BG-Sentinel (diameter: 36 cm / 14 inches; height: 40 cm / 1.3 feet) is a simple suction trap (Fig. 11) originally designed to collect mosquitoes. Due to its white coloured packing, however, it also proved to be attractive to a large number of pollinators (Grootaert & Dekoninck, in litt.). The trap is essentially a collapsible pop-up container with a white gauze cover, and an inlet at the top. Air is sucked into the trap through a black catch pipe at the top by an electrical fan, drawing approaching mosquitoes and other insects into a collecting bag. The air then exits the trap through the white gauze, generating ascending currents (Fig 11, red arrows). These are similar to convection currents produced by a human host, both in its direction, its geometrical structure, and due to the addition of artificial human skin odours (BG-Lure), also in its chemical composition (BioGents, 2007). Insects are gathered in the collecting bag and dried. The nylon collecting bag can be placed in a cooler and later on transferred to a deep freezer. Alternatively, dried insects can be sorted and pinned immediately or transferred to an alcohol solution. Specimens collected in this way prove suitable for DNA sequencing, even when collected after one week of sampling, which is a major advantage.

Recommendations (for the BG-Sentinel trap):

- A roof should be provided in (expectedly) rainy weather to cover the trap;
- Samples are best removed every two days to prevent damage to the dried insects by large live insects; this can be combined with replacing of the batteries.

***Restrictions*:**

- While using a suction trap to investigate vegetation or the litter or soil layer, plant material and debris is collected which cause damage to the collected invertebrates;
- The working capacity of the batteries of the BG-Sentinel type is two days.

Fig. 10. The BG-Sentinel suction trap was originally designed to collect mosquitoes. The arrows indicate the convection stream with yellow arrows corresponding with the air that is sucked in, and red arrows showing the air stream carrying the odours of the lure out of the trap. Due to its white colour many pollinators are collected. (Photo by Wouter Dekoninck).

4. References

ANONYMOUS 2004. http://www.nhm.ac.uk/researchcuration/research/projects/chalcidoids/collecting1.html

BAILLOT, S. & TRÉHEN, P. 1974. Variations de l'attractivité des pièges colorés de Moericke en fonction de la localisation spatio-temporelle de l'émergence, des comportements sexuels et des phases de dispersion de quelques espèces de diptères. *Annales de Zoologie, Ecologie animale* 6: 575-584.

BIOGENTS, A.G. 2007. Manual for the BG-Sentinel: an innovative trapping system for mosquitoes and other blood-sucking insects [Internet]. Regensburg, Germany BioGents AG. Available from: http://www.bg-sentinel.com/bilder/Manual_BG-Sentinel.pdf.

ELLIOTT, N.C., TAO, F.L., FUENTES-GRANADOS, R., GILES, K.L., ELLIOTT, D.T., GREENSTONE, M.H., SHUFRAN, K.A. & ROYER, T.A. 2006. D-vac sampling for predatory arthropods in winter wheat. *Biological Control* 38: 325-330

ERWIN, T.L. 1982. Tropical forests: their richness in Coleoptera and other arthropod species. *The Coleopterists' Bulletin* 36: 74-82.

GLEN, D.M. 1976. An emergence trap for bark-dwelling insects, its efficiency and effects on temperature. *Ecological Entomology* 1: 91-94.

GUEVARA, J. & AVILES, L. 2009. Elevational changes in the composition of insects and other terrestrial arthropods at tropical latitudes: a comparison of multiple sampling methods and social spider diets. *Insect Conservation and Diversity* 2: 142-152.

HUNTER, M.D. & PRICE, P.W. 1998. Cycles in insect populations: delayed density dependence or exogenous driving variables? *Ecological Entomology* 23: 216-222.

MACAULAY, E.D.M., TATCHELL, G.M. & TAYLOR, L.R. 1988. The Rothamsted Insect Survey '12-metre' suction trap. *Bulletin of Entomological Research* 78: 121-129.

MISSA O. 2000. Diversité et Hétérogénité de la faune des charançons (Coleoptera, Curculionidae) dans la canopée d'une forêt tropicale humide en Papouasie Nouvelle Guinée. Thèse de Docteur en Sciences, ULB: 158 pp.

MISSA, O., BASSET, Y., ALONSO, A., MILLER, S.E., CURLETTI, G., DE MEYER, M., EARDLEY, C., MANSELL M.W. & WAGNER, T. 2009. Monitoring arthropods in a tropical landscape: relative effects of sampling methods and habitat types on trap catches. *Journal of Insect Conservation* 13: 103-118.

POLLET, M. & GROOTAERT, P. 1987. Ecological data on Dolichopodidae (Diptera) from a woodland ecosystem. I. Colour preference, detailed distribution and comparison between different sampling techniques. *Bulletin de l'Institut royal des Sciences naturelles de Belgique, Entomologie* 57: 173-186.

POLLET, M. 1988. A technique for collecting quantitative data on Dolichopodidae and Empididae. *Empid and Dolichopodid Study Group Newsheet* 6: 5-7.

POLLET, M. & GROOTAERT, P. 1991. Horizontal and vertical distribution of Dolichopodidae (Diptera) in a woodland ecosystem. *Journal of Natural History* 25: 1297-1312.

POLLET, M., 1992. Impact of environmental variables on the occurrence of dolichopodid flies in marshland habitats in Belgium (Diptera: Dolichopodidae). *Journal of Natural History* 26: 621-636.

POLLET, M. & GROOTAERT, P. 1994. Optimizing the water trap technique to collect Empidoidea (Diptera). *Studia dipterologica* 1: 33-48.

POLLET, M. & GROOTAERT, P. 1996. An estimation of the natural value of dune habitats using Empidoidea (Diptera). *Biodiversity and Conservation* 5: 859-880.

POLLET, M. 2001. Dolichopodid diversity and site quality assessment of reed marshes and grasslands in Belgium (Diptera: Dolichopodidae). *Journal of Insect Conservation* 5: 99-116.

POLLET, M. 2002. Biodiversity patterns in Neotropical dolichopodid faunas (Diptera: Dolichopodidae). *Abstracts of the 5th International Congress of Dipterology.* Brisbane, Australia, 29 September - 4 October 2002: 194.

SMITH, M.A., WOODLEY, N.E., JANZEN, D.H., HALLWACHS, W., & HEBERT, P.D.N. 2006. DNA barcodes reveal cryptic host-specificity within the presumed polyphagous members of a genus of parasitoid flies (Diptera: Tachinidae). *Proceedings of the National Academy of Sciences of the United States of America* 103: 3657-3662.

STIREMAN, J.O. III, GREENEY, H.F. & DYER, L.A. 2009. Species Richness and Host Associations of Lepidoptera-Attacking Tachinidae in the Northeast Ecuadorian Andes. *Journal of Insect Science*, 39: 1-19.

STUBBS, A. & CHANDLER, P. 1978. *A dipterist's handbook*. *The Amateur Entomologist* 15: 255 pp.

TAUBER, C.A. & TAUBER, M.J. 1981. Insect Seasonal Cycles: Genetics and Evolution. *Annual Review of Ecology and Systematics* 12: 281-308.

VAN ASCH, M. & VISSER, M.E. 2007. Phenology of Forest Caterpillars and Their Host Trees: The Importance of Synchrony. *Annual Review of Entomology* 52: 37-55.

WILLIAMS, C.R., LONG, S.A., WEBB, C.E., BITZHENNER, M., GEIER, M., RUSSEL, R.C. & RITCHIE, S.A. 2007. *Aedes aegypti* population sampling using BG-Sentinel traps in north Queensland, Australia: statistical considerations for trap deployment and sampling strategy. *Journal of Medical Entomology* 44: 345-350.

Chapter 16

Recording insects by light-traps

by

Axel Steiner
Staatliches Museum für Naturkunde Karlsruhe
Erbprinzenstr. 13, 76133 Karlsruhe, Germany
Email: a-steiner@web.de

Christoph L. Häuser
Museum für Naturkunde Berlin - Leibniz-Institut für Evolutions- und Biodiversitätsforschung an der Humboldt-Universität zu Berlin
Invalidenstr. 43, 10115 Berlin, Germany
Email: christoph.haeuser@mfn-berlin.de

Abstract

Light-trapping is a general term which covers all methods of attracting and/or capturing nocturnal insects with lamps that usually have a strong emission in the ultraviolet range of the spectrum, *e.g.* mercury vapour lamps, black light lamps or fluorescent tubes. Nocturnal Lepidoptera (moths), Trichoptera and Ephemeroptera are the insect groups which can be collected most efficiently by light-trapping but many nocturnal species in several other orders are rarely recorded with other methods, *e.g.* some Coleoptera. There are various light-trap designs in common use, but they are all based on two general construction types. The advantages, limitations and performances of different trap types in relation to target group, study area, vegetation and weather conditions are briefly discussed with reference to relevant literature, and general recommendations for operations are given.

Keywords: monitoring, light trap design, light trap efficiency, abiotic factors, Lepidoptera

1. Introduction

The attraction of moths and other nocturnal insects to light is a well-known phenomenon and has been used for collecting nocturnal insects since the beginnings of scientific entomology in the 18th century. Light-trapping has become a general term which refers to all methods of attracting nocturnal insects with lamps or artificial light sources, whether they are actually connected to a trap or just being operated in front of walls or other reflective surfaces where incoming insects are then recorded or collected manually. The first purpose built devices which could be termed actual light-traps were used by the Romans in the 1st century AD (Morge, 1973; Steiner, 1991; Beavis, 1995).

While the physiological background of the attraction to light is still under discussion (see *e.g.* Hsiao, 1972, 1973; Baker & Sadovy, 1978; Sotthibandhu & Baker, 1979), attracting nocturnal insects with ultraviolet light is now in general use and presents the most effective collecting method for nocturnal species of the orders Lepidoptera, Trichoptera, and Ephemeroptera, but also for many species of Coleoptera, Hymenoptera, Diptera, Neuroptera s.l., Orthoptera, and some other insect groups. Automatic light traps have also become standard equipment for insect pest control and pest management but will not be considered here further, as these devices are purely designed to kill or even destroy the insects attracted and thereby preclude any scientific application.

The main advantage of light-trapping is the large number of species which can be recorded during a relatively short period. In Europe, for example, this can amount to 200 or more species of Lepidoptera in a single night under favourable conditions with the number of individuals running into the thousands. In the tropics the total count both of individuals and of species can be even much higher, often exceeding the available capacity for recording or collecting. On the other hand, light-trapping is still a selective method and not all taxa of a given group (family, genus) are attracted to light with the same efficiency, and females of many species are less attracted than males or not at all. For ecological studies it is sometimes seen as a drawback that light-trapping is an attraction method and it is thus not possible to directly link the species recorded to their respective (larval) habitats.

Overall there are two main approaches in the use of light traps. The qualitative approach aims at maximizing record and/or catch efficiency. For faunistic purposes, and for inventorying or assessing larger areas, it is usually preferable to use high-powered lights (*e.g.* 125 W lamps) and to chose sampling sites for maximum effect and across habitat-types, such as ridge tops, forest edges, etc. For ecological and habitat-related studies which require standardized comparisons and often target habitat- or niche-specific species it is better to use low-powered lamps (*e.g.* 8 W fluorescent tubes) placed well inside the target habitats (Wirooks, 2005).

2. Lamp types

While insects are attracted in a lesser degree to open fire, oil lamps, paraffin lamps, kerosene lamps and other light sources, the most effective lamps are those with a high emittance in the UV part of the spectrum. For most nocturnal insects the attractive part of the light spectrum lies in the ultraviolet range, somewhere between 350 nm and 550 nm (Cleve, 1954; Dufay, 1964, 1965; Mikkola, 1972; Hartstack, 1979) though spectral sensitivity varies from species to species; in a number of nocturnal Lepidoptera taxa Eguchi *et al.* (1982) reported peak sensitivities especially around 440-480 nm, and around 500-540 nm.

For field work, however, the choice of lamp type is more often determined by the actual field conditions than purely by scientific considerations. If there is access to the electricity network or if a portable generator is available, mercury vapour lamps, black-light lamps or blended (mixed light) lamps are usually the best choice because their emittance in the UV range is higher than that of standard household light bulbs (tungsten bulbs). If weight and size are an issue or in field situations without a mains power supply, fluorescent tubes are a perfect alternative which can be run from rechargeable 12 V batteries.

2.1. Mercury vapour and other UV lamps

High pressure mercury vapour lamps come in several sizes of which the 80 W and 125 W versions are those most used by entomologists. A larger 250 W version (which is no longer manufactured) is even more effective but also more trying for the human eye. All of those lamps require a separate electronic ballast (choke) to be inserted between the lamp and the power outlet. There are also 80 W versions which can be run without a ballast. The so-called black-light bulbs (125 W) produce almost no visible light; for the human eye they seem dark blue. They are thus suitable for situations where bright light is undesirable, *e.g.* in residential areas. For many groups, the 160 W blended (mixed light) lamps are less effective than the 125 W mercury vapour lamps but require no external ballast. There is also a 160 W black light bulb available, which does not need a ballast. Details can be obtained from manufacturers or from entomological suppliers via the internet.

2.2. Fluorescent tubes

The low pressure fluorescent tubes or neon tubes generally produce a bluish light and are available in a range of sizes in different lengths: 6 W (22.5 cm), 8 W (30 cm), 15 W (45 cm), 20 W (60 cm). Two special types emitting UV light are commonly used for light-trapping: the so-called "super actinic" tubes producing pale blue light, and "black light" tubes which are comparable to the black-light bulbs and are virtually invisible from a distance. While fluorescent tubes can also be operated with a voltage converter from a generator or mains power supply, in the field they are best directly run from 12 V rechargeable batteries.

A number of studies have compared the relative performance of different lamp types and their attraction on various insect orders (Williams, 1951; Bretherton,

1954; Williams *et al.*, 1955; Cleve, 1954, 1966, 1967; Lam and Stewart, 1969; Mikkola, 1972; Taylor and Brown, 1972; Taylor and French, 1974; Blomberg *et al.*, 1976; Walker and Galbreath, 1979; Leinonen *et al.*, 1998).

3. Trap design

In general, all lamps can be used without any trap or collecting vessel and incoming insects can be recorded or collected manually (Figs 1-5). This is often practised for faunistic studies and in cases when only particular species or specimens are of interest, especially if higher numbers of insects are likely to be attracted which would unnecessarily be collected by a trap or damage the desired specimens inside the collecting container. The lamp is best placed in front of a vertical white sheet, a wall or any other substrate which serves as a good reflector and also allows insects to settle near the lamp. Placing the lamp inside a larger gauze cylinder has the advantage that insects can be similarly attracted from all directions and that the lamp cannot be reached directly by incoming insects (see Figs 4 & 5). The simplest method is still to hang the lamp above a sheet lying on the ground

Fig. 1. Personal light-trapping. The sheet method. A white linen sheet mounted on a frame of aluminium poles, with two battery-powered 15 W fluorescent tubes, one actinic, one black.(Photo by A. Steiner).

Fig. 2. Personal light-trapping. A 125 W mercury vapour lamp and a sheet in a tropical rainforest. Note necessity of rain protection. (Photo by A. Steiner).

Fig. 3. Personal light-trapping. A simple set-up: A black-light bulb in a wire-frame housing at the white wall of a house. (Photo by A. Steiner).

Fig. 4. Personal light-trapping. Two battery-powered 15 W fluorescent tubes in a gauze cylinder ("tower"). (Photo by A. Steiner).

Fig. 5. Personal light-trapping. A combination of a 125 W mercury vapour lamp and two 15 W actinic fluorescent tubes in a gauze cylinder. (Photo by A. Steiner).

For actual light traps, there is a variety of individual designs in use and a vast literature available about the subject. Most designs, however, are based on the following components.

Basic features:

- Lamp
- Funnel
- Collecting container or receptacle

Additional features:

- Rain protection for light bulb
- Rain drainage
- Baffles or deflector shields
- Photoelectric switch
- Anaesthetic or killing agent

The lamp is the attractant. It is placed above or in front of a funnel which directs the insects into a collecting container, jar or receptacle. In addition, the trap can be provided with a range of useful features like a roof structure to protect the light bulb from rain and to prevent leaves, twigs, etc. from falling into the funnel. Alternately or additionally a rain drainage system can be installed, usually consisting of a small drainage funnel below the main funnel entry. A simple hole in the bottom of the trap collecting container covered with fine gauze is sometimes useful, but if a killing agent heavier than air is used the opening of the drainage funnel has to be raised above the bottom of the container.

A number of deflecting shields or baffles - usually two to four - made from Plexiglas, plastic or metal can be arranged around the lamp so that at least the larger, heavier, and faster-flying specimens fall into the funnel when hitting the baffles while circling the lamp.

Nowadays a photoelectric cell is an almost universal component of light traps. It allows the trap to be brought into the field at any time of day; the light-sensitive cell (the sensitivity can be regulated) switches the light on at dusk and off at dawn.

An anaesthetic or killing agent is often used inside the trap container to avoid damage of the specimens. Chemicals like chloroform ($CHCl_3$) or tetrachloroethane (1,1,2,2-tetrachloroethane, $C_2H_2Cl_4$) are left to evaporate from a vial or small bottle by means of a wick, whereas the often used ethyl-acetate is much less useful as it evaporates too quickly. Note that openings at the bottom of the trap have to be avoided (see caution about rain drains above).

Special features:

- Fan
- Wire mesh trays for separating insects according to size

When the trap is run without an anaesthetic it can be helpful to place a small fan inside the trap container to simulate wind which keeps the specimens inactive. Some trap designs include wire mesh or trays for automatically sorting specimens by size so that smaller insects reach the bottom trays and are less susceptible to damage by larger specimens (Common & Upton 1964; Vaishampayan, 1985a, b).

Fig. 6. Trichoptera and Lepidoptera at a gauze cylinder (Photo by A. Steiner).

Figures 7-8 illustrate two different trap designs. More information about specific designs including detailed drawings can be obtained from the literature, *e.g.* Muirhead-Thomson (1991), Fry & Waring (2001), or from individual supplier websites. For some examples of individual trap designs: Rothamsted light trap (Williams 1936, 1948; Taylor & Brown, 1972); Robinson light trap (Robinson & Robinson, 1950); Jermy trap (Jermy, 1961); Common trap (Common, 1960; Common & Upton, 1964); Heath trap (Heath, 1965). In all light traps, design significantly influences the catch especially with regard to the relative composition of different taxa, which can also be used to collect selectively specific target taxa (*e.g.*, Denmark, 1964; Lam & Stewart, 1969; Farrow, 1974; Sutton, 1979; Intachat & Woiwod, 1999).

Fig. 7. A hanging light-trap without rain-cover, showing three baffles around a 6 W actinic tube, a collapsible funnel made of thick plastic film, and a bucket as container. (Photo by A. Steiner).

Fig. 8. The same trap, disassembled. Top right: container. Right: actinic tube inside a Plexiglas cover with cable. The electronics are housed in the black top cap. Left: Plexiglas baffles and lower part of funnel. Centre: collapsible funnel with stabilising ring, screws for fastening baffles to tube housing, rubber ring for fastening lower part of funnel to container lid. (Photo by A. Steiner).

Fig. 9. A ground light-trap with a rain cover and three baffles around an 8 W black-light tube. The container is a commercially available plastic box. The black dot on the small grey box containing the electronics is the photoelectric cell. (Photo by A. Steiner).

4. Distance of light-response in nocturnal insects

In the past there was much difference of opinion about the effective range of attraction of light sources. More or less speculative values were given from around 1 m to 50 m (Daniel, 1952) or even up to 1.000 m (Koch, 1958). Various experimental studies – with different light sources and different study groups – have yielded effective distances of 3 m to 250 m (Bowden, 1982; Muirhead-Thomson, 1991). An unresolved question is whether specimens which obviously came from far outside the sampling habitat were attracted directly over a great

distance or were on a dispersal flight and at some point entered the effective range of the lamp and only then became attracted (which is more probable).

- Mark-release-recapture experiments of Sphingidae (Lepidoptera) around a 125 W mercury vapour lamp in tropical ecosystems (Borneo) suggested attraction radii (for 50% return rate within 5 minutes) of generally below 30 m (Beck & Linsenmair, 2006).
- Experiments with caged moths showed that a 15 W black light tube at a distance of 6.1 m caused 75% of *Heliothis zea* moths (Lepidoptera: Noctuidae) to move towards the light. At a distance of 69 m this response was shown by 10% of the moths. By extrapolation the maximal range of attraction was determined as 60-90 m. In *Manduca sexta* (Lepidoptera: Sphingidae) 48% of individuals showed a positive response at a distance of 4.6 m from the light source; the maximal range of attraction was determined as 120-135 m (Stewart *et al.*, 1967).
- In a similar experimental setup the threshold of attraction was calculated to be 200-250 m for *Spodoptera littoralis* (Lepidoptera: Noctuidae) (Plaut, 1971).
- Physiological studies on the eyes of *Heliothis zea* and *Heliothis virescens* (Lepidoptera: Noctuidae) showed that 15 W blacklight tubes can trigger sensory responses from distances between 31 m and 250 m (Agee, 1972).
- Under the assumption that nocturnal insects react to wavelengths of 500-600 nm, Bowden & Church (1973) calculated the radius around a 125 W mercury vapour lamp within which the brightness of the light source is higher than the background brightness. They obtained values between 35 m (in full moon nights) and 520 m (without moonlight). On a similar basis Dufay (1964) reached results of 50 m to 700 m for another type of 125 W MV lamp, while Nowinszky *et al*. (1979) calculated distances of between 20 m (full moon) and 300 m (no moon) for a 100 W Argon bulb.

5. The role of abiotic factors

There is an abundant literature on the many abiotic and other factors which influence light trap efficiency and sample size. We can only give a basic overview and provide references of more detailed studies.

5.1. Temperature

Ambient air temperature seems to be the most important single factor influencing insect flight activity and thus the catch (Williams, 1940; Daniel, 1952; Hosny, 1959; Taylor, 1963; Pulliainen, 1965; Hanna & Atries, 1969a; Persson, 1971, 1976; Kurtze, 1974; Hanna & Hamad, 1975b; Blomberg *et al.*, 1978; Morton *et al.*, 1981; Dent & Pawar, 1988; McGeachie, 1989). Generally speaking, the higher the temperature the more insects are active, which usually translates into highest activity rates during the first hours after sunset. Rapid cooling during the night will cause inactivity sooner than slow cooling. In temperate climates cloud cover at night means less rapid cooling and thus a longer activity period of insects. Temperature dependency, of course, varies with the climate zone a

species inhabits: boreal and alpine species are adapted to lower temperatures than thermophilic, subtropical or tropical species, and specialist species having their peak activity during periods of comparatively low temperature can be found in all biomes, including the famous "winter moths" and "winter midges" of northern hemispheres.

5.2. Moonlight and starlight

Lunar periodicity plays an important role in catch efficiency and has been the subject of numerous studies (Williams, 1936; Williams & Singh, 1951; Hosny, 1959; Dufay, 1964, 1965; Hanna & Atries, 1969b; Persson, 1971, 1976; Bowden, 1973, 1981, 1982, 1984; Bowden & Church 1973; Hartstack *et al.*, 1973; Kurtze, 1974; Bowden & Morris, 1975; Hanna & Hamad, 1975a; Douthwaite, 1978; Nowinszky *et al.*, 1979; Morton *et al.*, 1981; Vaishamapayan and Verma 1982; Danthanarayana, 1986; Taylor, 1986; Dent & Pawar, 1988; McGeachie, 1989; Nag & Nath 1991). In short, the stronger the moonlight is, the less attraction a lamp has to insects. The ratio between catch in new moon nights and catch in full moon nights has been given as 2,67: 1 (Williams, 1940; a 4-year study in England) and as 2.59: 1 (Nowinszky *et al.*, 1979; 14 years of light-trapping in Hungary). While it was once suspected that insect activity in general might be lower in moon nights, it has since been shown that lamp attraction is weaker. In fact insect activity seems to be higher in bright, moonlit nights as indicated by comparisons of light-trapping with other methods such as suction traps (Bowden, 1981) and pheromone traps (Dent & Pawar, 1988). When insect activity actually diminishes in moon nights this is usually due to other negative weather factors, especially rapidly falling temperatures as commonly observed in clear nights. In subarctic regions, however, the naturally bright summer nights make lamps less attractive to insects (Blomberg *et al.*, 1978).

The relationship of background brightness (light emitted by moon and stars) and catch efficiency has been expressed in the formula:

$$\text{catch} = \text{constant} \times \sqrt{W/I}$$

where W represents lamp brightness and I is background brightness. With a constant lamp brightness there is:

$$\text{catch} = \text{constant} \times \sqrt{1/I}$$

Other weather factors can significantly influence this ratio (Bowden & Church, 1973; Bowden, 1981, 1982), while cloud cover mitigates the competing effects of moon light.

5.3. Wind

Wind speed is another important factor affecting insect activity and especially flight (Hosny, 1955, 1959; Williams, 1961; Dufay, 1964, 1965; Brown, 1970; Persson, 1971, 1976; Kurtze, 1974; Hanna & Hamad, 1975b; Douthwaite, 1978; Morton *et al.*, 1981; Tucker, 1983; Dent & Pawar, 1988; McGeachie, 1989). In stronger wind there is less insect activity: most species cease flying as soon as

they cannot any longer maintain a directional flight. The critical wind speed varies according to size and strength: larger moths (Noctuidae) cease flying to lamps at wind speeds of 10.8-13.8 m/s, smaller Diptera, Tipulidae, Limnobiidae, and Chironomidae at 8.0-10.7 m/s, Psychodidae and Trichoceridae at 6.7-9.4 m/s, and Ceratopogonidae and Cecidomyiidae at 3.4-5.4 m/s (Kurtze, 1974). A marked reduction of catch occurs at 3-4 m/s (Douthwaite, 1978) and at 4 m/s (Dent & Pawar, 1988). The highest catch rates, however, are not recorded at calm but at wind speeds between 1 and 3 m/s (Hosny, 1955; Douthwaite, 1978; Dent & Pawar 1988).

5.4. Precipitation, air humidity, and fog

Strong rainfall can reduce or prevent insect activity, especially for smaller species, while most insects are usually indifferent to light rain (drizzle, spray) unless it coincides with a drop in ambient temperature. Under certain conditions, *e.g.* in dry or semiarid areas but also in tropical regions with a pronounced rainfall seasonality, rain can induce eclosion and stimulate activity (Williams, 1940; Daniel, 1952; Hosny, 1955, 1959; Pulliainen, 1965; Harling, 1968; Brown *et al.*, 1969; Persson, 1971; Kurtze, 1974; Douthwaite, 1978; Tucker, 1983). In the tropics rain often considerably increases light trap attractivity, often leading to unusual and rare records. For running a light during tropical rain, the lamp or trap is best protected by a larger roof, which can be easily constructed with some canvas or tarpaulin (Malicky, 2002; see also Fig. 2). In addition, some drainage provisions around the position of the trap are often a helpful measure (*e.g.*, Diehl, 2001).

In temperate conditions, high air humidity can also promote insect activity unless combined with cooling. Fog in combination with falling temperatures or fog which forms in valley bottoms, basins, and wetlands, strongly reduces insect activity. Dewfall is usually a result of cooling and coincides with reduced activity. Drifting clouds and fog on slopes or in the mountains need not to lead to negative results; in certain situations they actually seem to intensify the attraction of light traps (Daniel, 1952; Hosny, 1955, 1959; Hanna & Atries, 1969a; Kurtze, 1974; Hanna & Hamad, 1975b; Esche, 1992).

5.5. Air pressure

It is sometimes said that falling air pressure improves general insect activity, *e.g.* before thunderstorms (Haase, 1929; Allan, 1947; Hosny, 1955; Lederer, 1959) while other studies claim there is no recognisable influence of air pressure (Dufay, 1964, 1965). Without quantitative studies or experimental evidence at hand, however, we also have experienced many times the highest attraction of light traps at times just before the onset of thunderstorms or heavy rainfall, both in temperate and especially under tropical conditions; whether it is specifically air pressure or other factors related to the imminent change of weather conditions which lead to high levels of insect activity remains unclear, but such situations are usually always advantageous for light-trapping.

In addition to climate and weather related factors, several locality-related conditions also play an important role in determining the most productive sites for light traps.

Forest vs. open country

Inside forests the negative effect of moonlight is less dramatic. Bowden (1982) studying trapping data from Rothamsted (England, U.K.) noted a catch ratio of *Noctua pronuba* (Lepidoptera: Noctuidae) between open habitats and forests of 1 : 3.7. Temperature change, especially nocturnal cooling is often less marked in forests, and winds are weaker. On the other hand light has a larger radius in open areas (Hosny 1955, 1959; Bowden, 1982), and results are significantly different between light traps placed in the understorey and in the forest canopy, especially in the tropics (Schulze *et al.*, 2001; Beck & Linsenmair, 2006).

Wind direction

Most insects prefer to fly against the wind when looking for food or locating females. Exceptions are migrating specimens which use wind currents and fly with the wind (Brown *et al.*, 1969; Brown, 1970). When smaller areas are to be studied it is thus advantageous to place traps at their windward side.

Terrain structure and landscape

Many insects prefer to fly upslope, also at night. Lights placed on slopes or hilltops may control a larger area; even considering that a lamp's direct effective range of attraction may be quite small, there is a higher chance that more specimens reach the neighbourhood of the trap. The landscape (and vegetation) surroundings of the light trap location also greatly influence the results, *e.g.* by offering protection from or providing exposure to local wind currents and other weather factors, and through different local microclimatic conditions, including varying albedo properties. Cold air often accumulates in even small depressions and valley bottoms, while certain terrain structures such as bare rocks can absorb heat during the day and emit part of that radiation at night. Selecting the exact placement of a light trap should also take these factors into account.

6. Concluding remarks

For any new light-trapping project, the choice of the equipment to be used is clearly an important initial step. Aside from the relevant technical and biological parameters that different lamps and trap constructions entail, the final choice should also consider more practical criteria, such as weight and transportability, durability under field conditions, and availability and cost of spare parts or repairs. It should be kept in mind that there exists no overall most effective or "best" lamp type nor "standard" light trap construction or design; all types and makes of light traps are differently selective in one way or another, and the final choice should be determined by the exact question(s) and goals to be pursued by the study. Although most equipment discussed here works well for most insect

taxa and many different habitats, no one type of light trap will equally attract all taxa. For aiming at a comprehensive inventory such as an ATBI of a local fauna or a community of different taxa, it is therefore advisable to employ a number of different lights and trap designs, if at all possible.

With standardization of methods being a requirement for many scientific approaches in order to allow for comparable and/or repeatable collection of data, especially from ecology, light-trapping provides a clear method of choice for many entomological studies. While standardization can be easily achieved for the equipment and light-trapping regime, other factors relevant for the results are much more difficult to compare or even standardize, even if the availability of fully automatic light traps allows reducing the influence of the "human factor" to a certain degree. Apart from the important effects of weather, moonlight and other factors discussed above, the exact placement of a trap in the field remains the overall most difficult and perhaps still influential parameter in making light-trapping data fully comparable, especially for highly structured habitats and landscapes such as forests and mountains. As indicated above and experienced many times, the precise placement of the light in relation to its surroundings greatly impacts the results, with sometimes a few feet or meters distance already leading to noticeably different catches. Especially for manually operated lights, finding the "best" precise location is almost always the biggest challenge in the field, for which personal experience often still provides the best guidance. All these methodological challenges should provide additional incentives for the precise recording and documenting any light-trapping session, especially for exact geographic coordinates, time, and weather conditions, which should be a common standard under all light-trapping circumstances.

7. Tips and hints – some "do-s and don't-s"

- The higher a lamp/trap is placed above the ground, the larger is the area it controls. Be sure to have sufficient possibilities to raise the light and/or trap above ground on site (*e.g.*, by carrying poles or other equipment).
- Stronger light generally means higher attraction (more specimens/species), but some species prefer to settle at some distance from bright lamps. It is often helpful to carefully check the perimeter around such a lamp to find those species.
- Small moths and other insects with a gentle flight often come to rest on the baffles of a trap or in the vegetation nearby and do not enter the collecting container. Traps should therefore be checked well before sunrise, before these specimens fly away or are eaten by birds and other predators. It is helpful to place the trap on a large white sheet or a similar background that makes it easier to find those specimens.
- Before placing light traps for longer-term studies in the field, check and record the microclimatic conditions at night at the exact location, particularly with regard to air temperature, wind strength, and wind direction.
- When using a trap without a killing agent, the container needs to be filled with materials to provide sufficient resting space for the specimens. Many authors

recommend using egg cartons, which however we find very difficult to extract resting specimens from. Instead, we recommend using rough, slightly crumpled paper, because this is easier to handle and can be more readily straightened to box specimens.

- When running light traps with a killing agent especially for specific, limited questions, try to ensure that the by-catch is also kept for / used by other researchers; all specimens collected with accurate data can be of value!
- Do not look directly into a mercury vapour lamp. Although the UV radiation from MV lamps is considered not harmful for the human eye, individual sensitivity varies and emission from strong MV lamps can be irritating.
- When going into the field, always carry sufficient torches and other additional light sources along; if for no other reason, setting up and taking down light trap equipment at night can be quite difficult without sufficient torches at hand.
- Always take some basic tool kit (screwdriver, pincer, small knife, electrical tape) along when light-trapping; equipment gets easily damaged under field conditions, and it is advantageous to be able to do basic repairs on site.

8. Acknowledgements

For valuable help and support related to the preparation of this paper we would like to thank in particular Daniel Bartsch, Carlos Monje, Robert Trusch, Ludger Wiroks and Andreas Zwick. In addition, we benefited greatly from the knowledge and field experiences shared by many friends and colleagues over many years for which we are equally grateful.

9. References

AGEE, H.R. 1972. Sensory response of the compound eye of adult *Heliothis zea* and *H. virescens* to ultraviolet stimuli. *Annals of the Entomological Society of America* 65: 701-705.

ALLEN, P.B.M. 1947. *A Moth-Hunter's Gossip.* London (Watkins & Doncaster): 269 pp.

BAKER, R.R. & SADOVY, Y. 1978. The distance and nature of the light-trap response of moths. *Nature* 276: 818-821.

BEAVIS, I.C. 1995. The first light trap, 1st century AD. *The Entomologist's Record and Journal of Variation* 197: 155.

BECK, J.F. & LINSENMAIR, K.E. 2006. Feasibility of light-trapping in community research on moths: Attraction radius of light, completeness of samples, nightly flight times and seasonality of Southeast-Asian hawkmoths (Lepidoptera: Sphingidae). *Journal of Research on the Lepidoptera* 39: 18-37.

BLOMBERG, O., ITÄMIES, J. & KUUSELA, K. 1978. The influence of weather factors on insect catches in traps equipped with different lamps in northern Finland. *Annales Entomologici Fennici* 44: 56-62.

BOWDEN, J. 1973. The influence of moonlight on catches of insects in light-traps in Africa. Part I. The moon and moonlight. *Bulletin of Entomological Research* 63: 113-128.

BOWDEN, J. 1981. The relationship between light- and suction-trap catches of *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae), and the adjustment of light-trap catches to allow for variation in moonlight. *Bulletin of Entomological Research* 71: 621-629.

BOWDEN, J. 1982. An analysis of factors affecting catches of insects in light-traps. *Bulletin of Entomological Research* 72: 535-556.

BOWDEN, J. 1984. Latitudinal and seasonal changes of nocturnal illumination with a hypothesis about their effect on catches of insects in light-traps. *Bulletin of Entomological Research* 74: 279-298.

BOWDEN, J. & CHURCH, B.M. 1973. The influence of moonlight on catches of insects in light-traps in Africa. Part II. The effect of moon phase on light-trap catches. *Bulletin of Entomological Research* 63: 129-142.

BOWDEN, J. & MORRIS, M.G. 1975. The influence of moonlight on catches of insects in light-traps in Africa. III. The effective radius of a mercury-vapour light-trap and the analysis of catches using effective radius. *Bulletin of Entomological Research* 65: 303-348.

BRETHERTON, R.F. 1954. Moth traps and their lamps: an attempt at comparative analysis. *Entomologist's Gazette* 5: 145-154.

BROWN, E.S. 1970. Nocturnal insect flight direction in relation to the wind. *Proceedings of the Royal Entomological Society of London, Series A*, 45: 39-43.

BROWN, E.S., BETTS, E. & RAINEY, R.C. 1969. Seasonal changes in distribution of the African armyworm, *Spodoptera exempta* (Wlk.) (Lep., Noctuidae), with special reference to eastern Africa. *Bulletin of Entomological Research* 58: 661-728.

CLEVE, K. 1954. Einfluß der Wellenlänge des Lichtes auf den Lichtfang der Schmetterlinge. *In:* TITSCHAK, E. (Ed.). *Deutscher Entomologentag in Hamburg 30. Juli bis 3. August 1953*, Jena (Fischer): 107-113.

CLEVE, K. 1966. Das Sternenlicht und dessen vermutliche Wahrnehmung durch nachts fliegende Schmetterlinge. *Deutsche Entomologische Zeitschrift N. F.* 13: 359-375.

CLEVE, K. 1967. Das spektrale Wahrnehmungsvermögen nachts fliegender Schmetterlinge (Lep.). *Nachrichtenblatt der Bayerischen Entomologen* 16: 33-53.

COMMON, I.F.B. 1960. A transparent light trap for the field collection of Lepidoptera. *Journal of the Lepidopterists' Society* 13: 57-61.

COMMON, I.F.B. & UPTON, M.S. 1964. A weather-resistant light trap for the collection of Lepidoptera. *Journal of the Lepidopterists' Society* 18: 79-83.

DANIEL, F. 1952. Praxis des Nachtfangs mit Licht. *Nachrichtenblatt der Bayerischen Entomologen* 1: 44-46, 51-54, 61-63, 67-68.

DANTHANARAYANA, W. 1986. Lunar periodicity of insect flight. *In:* DANTHANARAYANA, W (Ed.). *Insect Flight: Dispersal and Migration*. Springer: 88-119.

DENMARK, H.A. 1964. Evaluation of an insect light trap designed to separate beetles and moths. *Journal of the Lepidopterists' Society* 18: 1-10.

DENT, D.R. & PAWAR, C.S. 1988. The influence of moonlight and weather on catches of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) in light and pheromone traps. *Bulletin of Entomological Research* 78: 365-377.

DIEHL, E.W. 2001. Moderner Lichtfang unter besonderer Berücksichtigung tropischer Verhältnisse. *Entomologische Zeitschrift* 111: 308-311.

DOUTHWAITE, R.J. 1978. Some effects of weather and moonlight on light-trap catches of the army-worm, *Spodoptera exempta* (Walker) (Lepidoptera: Noctuidae), at Muguga, Kenya. *Bulletin of Entomological Research* 68: 533-542.

DUFAY, C. 1964. Contribution à l'étude du phototropisme des lépidoptères noctuides. *Annales des Sciences Naturelles - Zoologie et Biologie Animale, Paris, 12e série* 6: 281-406.

DUFAY, C. 1965. Étude du phototropisme des Lépidoptères Noctuides. Applications aux chasses à la lumière. *Alexanor* 4: 81-88, 131-136.

EGUCHI, E., WATANABE, K., HARIYAMA, T. & YAMAMOTO, K. 1982. A comparison of electrophysiologically determined spectral responses in 35 species of Lepidoptera. *Journal of Insect Physiology* 28: 675-682.

ESCHE, TH. 1992. Konkurrieren Nachtschmetterlinge um Blüten? Untersuchungen zu Nischentrennung und Bestäubungseffektivität. Dissertation. University of Freiburg: 194 pp.

FARROW, R.A. 1974. A modified light trap for obtaining large samples of night-flying locusts and grasshoppers. *Journal of the Australian Entomological Society* 13: 357-360.

FRY, R. & WARING, P. 2001. A Guide to Moth Traps and their Use. *The Amateur Entomologist* 24: iv, 1-68.

HAASE, J. 1929. Die Lichtfalle, ein Hilfsmittel zur Erforschung der Lokalfauna. *Internationale Entomologische Zeitschrift [Guben]* 23: 89-107.

HANNA, H.M. & ATRIES, I.E. 1969a. The flight activity of certain nocturnal Lepidoptera in relation to temperature and humidity. *Bulletin de la Société Entomologique d'Egypte* 53: 1-6.

HANNA, H.M. & ATRIES, I.E. 1969b. The effect of moonlight on certain nocturnal Lepidoptera. *Bulletin de la Société Entomologique d'Egypte* 53: 7-12.

HANNA, H.M. & HAMAD, N.E.F. 1975a. Lunar periodicity and flight activity of nocturnal Lepidoptera at different levels. *Bulletin de la Société Entomologique d'Egypte* 59: 17-27.

HANNA, H.M. & HAMAD, N.E.F. 1975b. Effect of climatic factors on flight activity of moths of certain cotton pests. *Bulletin de la Société Entomologique d'Egypte* 59: 39-47.

HARLING, J. 1968. Meteorological factors affecting the activity of night-flying Macro-Lepidoptera. *The Entomologist* 101: 83-93.

HARTSTACK, A.W. 1979. Light sources, trap design and other factors affecting moth catch. *In*: RABB, R.L. & KENNEDY, G.G. (Eds). *Movement of highly mobile insects: concepts and methodology in research*. Raleigh: 232-241.

HEATH, J. 1965. A genuinely portable MV light trap. *The Entomologist's Record and Journal of Variation* 77: 236-238.

HOSNY, M.M. 1955. Notes on the effect of some secondary environmental conditions on the activity of nocturnal Macrolepidoptera. *Bulletin de la Société Entomologique d'Egypte* 39: 297-314.

HOSNY, M.M. 1959. A review of results and a complete list of Macrolepidoptera caught in two ultra-violet light traps during 24 months, at Rothamsted, Hertfordshire. *Entomologist's Monthly Magazine* 95: 226-237.

HSIAO, H.S. 1972. *Attraction of moths to light and to infrared radiation*. San Francisco Press: 89 pp.

HSIAO, H.S. 1973. Flight paths of night-flying moths to light. *Journal of Insect Physiology* 19: 1971-1976.

INTACHAT, J. & WOIWOD, I.P. 1999. Trap design for monitoring moth biodiversity in tropical rainforests. *Bulletin of Entomological Research* 89: 153-163.

JERMY, T. 1961. Kártevö rovarok rajzásának vizsgálata fénycsapdákkal. *A Növényvédelem Idöszerü Kérdései* 2: 53-60.

KOCH, M. 1958. *Wir bestimmen Schmetterlinge*. Vol. 3. Neumann Radebeul/Berlin: 291 pp., 24 pls.

KURTZE, W. 1974. Synökologische und experimentelle Untersuchungen zur Nachtaktivität von Insekten. Synecological and experimental investigations on the night-activity of insects. *Zoologische Jahrbücher, Abteilung für Systematik* 101: 297-344.

LAM, J.J. & STEWART, P.A. 1969. Modified traps using blacklight lamps to capture nocturnal tobacco insects. *Journal of Economic Entomology* 62: 1378-1381.

LEDERER, G. 1959. Nahrungssuche und Nahrungsaufnahme der in der Dämmerung und in der Nacht fliegenden Lepidopteren. Grundlagen und Anleitung für das Köder- und Blütenfangverfahren. *Entomologische Zeitschrift* 69: 25-40, 42-56, 67-80, 94-100, 109-124, 126-132, 136-144.

LEINONEN, R., SÖDERMAN, G., ITÄMIES, J., RYTKÖNEN, S. RUTANEN, I. 1998. Intercalibration of different light-traps and bulbs used in moth monitoring in northern Europe. *Entomologica Fennica* 9: 37-51.

MALICKY, H. 2002. Ein Regendach für den Lichtfang. *Entomologische Zeitschrift* 112: 2-4.

MCGEACHIE, W.J. 1989. The effects of moonlight illuminance temperature and wind speed on light-trap catches of moths. *Bulletin of Entomological Research* 79: 185-192.

MIKKOLA, K. 1972. Behavioural and electrophysiological responses of night-flying insects, especially Lepidoptera, to near-ultraviolet and visible light. *Annales Zoologici Fennici* 9: 225-254.

MORGE, G. 1973. Entomology in the western world in antiquity and in medieval times. *In:* SMITH, R.F., MITTLER, T.E. & SMITH, C.N. (Eds). *History of Entomology*. Annual Reviews Inc. Palo Alto, CA: 37-80.

MORTON, R., TUART, L.D. & WARDHAUGH, K.G. 1981. The analysis and standardisation of light-trap catches of *Heliothis armiger* (Hübner) and *H. punctiger* Wallengren (Lepidoptera: Noctuidae). *Bulletin of Entomological Research* 71: 207-225.

MUIRHEAD-THOMSON, R.C. 1991. Trap responses of flying insects. The influence of trap design on capture efficiency. Academic Press London: xii + 287 pp.

NAG, A. & NATH, P. 1991. Effect of moon light and lunar periodicity on the light trap catches of cutworm *Agrotis ipsilon* (Hufn.) moths. *Journal of Applied Entomology* 111: 358-360.

NOWINSZKY, L., SZABÓ, S., TÓTH, G., EKK, I. & KISS, M. 1979. The effect of the moon phases and of the intensity of polarized moonlight on the light-trap catches. *Zeitschrift für Angewandte Entomologie* 88: 337-353.

PERSSON, B. 1971. Influence of light on flight activity of noctuids (Lepidoptera) in south Sweden. *Entomologica Scandinavica* 2: 215-232.

PERSSON, B. 1976. Influence of weather and nocturnal illumination on the activity and abundance of populations of Noctuids (Lepidoptera) in south coastal Queensland. *Bulletin of Entomological Research* 66: 33-63.

PLAUT, H.N. 1971. Distance of attraction of moths of *Spodoptera littoralis* to BL radiation, and recapture of moths released at different distances of an ESA blacklight standard trap. *Journal of Economic Entomology* 64: 1402-1404.

PULLIAINEN, E. 1965. Tutkimuksia yökkösten (Lep., Noctuidae) aktiivisuudesta ja Varkauden (PS) seudun yökkösfaunasta [Studies on the activity of noctuids (Lep., Noctuidae) and on the noctuid fauna in the vicinity of Varkaus in north Savo]. *Savotar* 5: 215-255.

ROBINSON, H.S. & ROBINSON, P.J.M. 1950. Some notes on the observed behaviour of Lepidoptera in flight in the vicinity of light sources, together with a description of a light trap designed to take entomological samples. *Entomologist's Gazette* 1: 3-20.

SCHULZE, C.H., LINSENMAIR, K.E. & FIEDLER, K. 2001. Understorey versus canopy: patterns of vertical stratification and diversity among Lepidoptera in a Bornean rain forest. *Plant Ecology* 153: 1533-1552.

SOTTHIBANDHU, S. & BAKER, R.R. 1979. Celestial orientation by the large yellow underwing moth, *Noctua pronuba* L. *Animal Behaviour* 27: 786-800.

STEINER, A. 1991. Der Einsatz von Lichtfallen bei der Schädlingsbekämpfung in der römischen Kaiserzeit, 1. Jahrhundert v. Chr. - 4. Jahrhundert n. Chr. (Insecta, Lepidoptera, Pyralidae). *Beiträge zur Entomologie* 41: 405-410.

STEWART, P.A., LAM, J.J. & HOFFMAN, J.D. 1967. Activity of tobacco hornworm and corn earworm moths as determined by traps equipped with blacklight lamps. *Journal of Economic Entomology* 60: 1520-1522.

SUTTON, S.L. 1979. A portable light trap for studying insects of the upper canopy. *Brunei Museum Journal* 4: 156-160.

TAYLOR, L.R. 1963. Analysis of the effect of temperature on insects in flight. *Journal of Animal Ecology* 32: 99-117.

TAYLOR, L.R. & BROWN, E.S. 1972. Effects of light-trap design and illumination on samples of moths in the Kenya highlands. *Bulletin of Entomological Research* 62: 91-112.

TAYLOR, L.R. & FRENCH, R.A. 1974. Effects of light-trap design and illumination on samples of moths in an English woodland. *Bulletin of Entomological Research* 63: 583-594.

TAYLOR, R.A.J. 1986. Time series analysis of numbers of Lepidoptera caught at light traps in East Africa, and the effect of moonlight on trap efficiency. *Bulletin of Entomological Research* 76: 593-606.

TUCKER, M.R. 1983. Light-trap catches of African armyworm moths, *Spodoptera exempta* (Walker) (Lepidoptera: Noctuidae), in relation to rain and wind. *Bulletin of Entomological Research* 73: 315-319.

VAISHAMPAYAN, S.M. 1985a. Factors affecting the light trap catches of insects with emphasis on design aspects. *In*: *Use of traps for pest/vector research and control*. Mohanpur, West Bengal (Proc. Nat. Seminar): 62-67.

VAISHAMPAYAN, S.M. 1985b. JNKVV trap SM-model-light trap for survey of insect pests and vectors. *In*: *Use of traps for pest/vector research and control*. Mohanpur, West Bengal (Proc. Nat. Seminar): 152-155.

VAISHAMPAYAN, S.M. & VERMA, R. 1982. Influence of moon light and lunar periodicity on the light trap catches of gram pod borer, *Heliothis armigera* (Hübner) moths. *Indian Journal of Entomology* 44: 206-212.

WALKER, A.K. & GALBREATH, R.A. 1979. Collecting insects at lights: a test of four types of lamp. *New Zealand Entomologist* 7: 83-85.

WILLIAMS, C.B. 1936. The influence of moonlight on the activity of certain nocturnal insects, particularly of the family Noctuidae, as indicated by a light-trap. *Philosophical Transactions of the Royal Society of London, Series B* 226: 357-389.

WILLIAMS, C.B. 1940. An analysis of four years captures of insects in a light-trap. Part II: The effect of weather conditions on insect activity; and the estimation and forecasting of changes in the insect populations. *Philosophical Transactions of the Royal Entomological Society of London, Series B* 90: 227-306.

WILLIAMS, C.B. 1948. The Rothamsted light trap. *Philosophical Transactions of the Royal Entomological Society of London, Series A* 23: 80-85.

WILLIAMS, C.B. 1951. Comparing the efficiency of insect traps. *Bulletin of Entomological Research* 42: 513-517.

WILLIAMS, C.B. 1961. Studies in the effect of weather conditions on the activity and abundance of insect populations. *Philosophical Transactions of the Royal Society of London, Series B* 244: 331-378.

WILLIAMS, C.B., FRENCH, R.A. & HOSNI SIC M.M. 1955. A second experiment on testing the relative efficiency of insect traps. *Bulletin Entomological Research* 46: 193-204.

WILLIAMS, C.B. & SINGH, B.P. 1951. The effect of moonlight on insect activity. *Nature* 167: 853.

WIROOKS, L. 2005. Die ökologische Aussagekraft des Lichtfangs. Eine Studie zur Habitatbindung und kleinräumigen Verteilung von Nachtfaltern und ihren Raupen. Havixbeck-Hohenholte Verlag Wolf & Kreuels: 320 pp.

Chapter 17

Flight interception traps for arthropods

by

Kees van Achterberg
Nationaal Natuurhistorisch Museum Naturalis
Postbus 9517, 2300 RA Leiden, the Netherlands
E-mail: achterberg@naturalis.nnm.nl

Patrick Grootaert
Royal Belgian Institute of Natural Sciences
Vautierstraat 29, B-1000 Brussels, Belgium
E-mail: Patrick.Grootaert@naturalsciences.be

Mark R. Shaw
National Museums of Scotland
Chambers Street, Edinburgh EH1 1JF, United Kingdom
Email: markshaw@xenarcha.com

Abstract

An overview is given of flight interception traps for arthropods since the discovery of the principle by R. Malaise. New and rare designs are described and suggestions for improvement and low cost improvisation are made. The effectiveness of the traps is discussed. An overview of killing agents and preservatives and their effects on specimens is given. Good and bad practices are listed and safety is discussed. Finally methods for preparing Hymenoptera and Diptera from alcohol are described.

Keywords: positive phototropism, Malaise trap, Schacht trap, window pane trap, placement of traps, Townes design, new designs, effectiveness, preparing Hymenoptera and Diptera, killing agents and preservatives, safety, ethics

1. General introduction

The aim of this chapter is to give an updated overview of the available flight interception traps, outlining their use, advantages and disadvantages, to facilitate the choice of the appropriate designs and to improve the efficiency and quality of the collecting of arthropods. The operation of interception traps is based on the interception of arthropods (in most cases insects) in the air by means of a vertical or oblique barrier. The subsequent reaction is positive if the intercepted insects are attracted by sunlight to fly or walk to the top of the trap ("positive phototropism"). If the insects try to hide by walking down or allowing themselves to fall down the reaction is negative ("negative phototropism"). Defined in this way Malaise traps are a kind of flight interception trap and the latter name should be applied to traps using both positive and negative phototropism. Flight interception traps can be used in any habitat where insects occur, but will be most efficient if corridors ("flyways") are present to be blocked by the trap. Their applicability is equal in temperate and tropical habitats, but abnormally low temperatures will lower trapping efficiency.

Collecting a large number of specimens from groups of no interest to the collector poses a potential ethical problem. Therefore, it is recommended that the unused portions are stored in central depositories (*e.g.*, national museums of natural history) at low temperature and in darkness. There the material can be made available to other specialists, who may extract the specimens of interest to their study. The problem of catching protected or flagship taxa is very rarely encountered, but in these cases either an extra mesh before the entrance of the collector could be used or the trap could be placed just outside the area where these taxa occur. Hardly anything has been published on the impact of flight interception traps on the local populations of insects. It has been assumed that at most about 20% of the Hymenoptera entering the trap is finally caught in the collector (late H.K. Townes, pers. comm.); as far as the authors are aware no estimates have been made for other traps. Experiments to ascertain the effects of trapping on insect populations would need careful design, and the results would be expected to be highly site and organism dependent. In publications the design of the trap (including the measurements of the sampling surface), the way it was used and the position of the trap related to the sun and vegetation should be stated.

It should be strongly borne in mind that many of the fluids used as preservatives are highly toxic to vertebrate animals that will frequently try to drink them, and this risk to wildlife as well as to domestic animals needs always to be minimised.

Placement of traps

According to Darling & Packer (1988) the effectiveness of a trap depends first of all on its placement within the micro-habitat, second on its design and last on the mesh-size. According to Matthews & Matthews (1983) the design is the most important, followed by its correct placement in the flyways of insects. Obviously, an effective placement is extremely important; poor placement may lower the

catches by more than 50% in the same micro-habitat (van Achterberg, unpublished data). Relatively small changes result in large differences in collection efficiency (Matthews & Matthews, 1983). In general the trap should be either blocking a corridor (*e.g.* a path in the forest) or placed perpendicular to a barrier (*e.g.* border of a forest, with the collecting head directed to the border and the sun). Malaise (1937) was already very aware of the importance of placement: "The chief difficulty in using this trap is to find a suitable place. A trap put up in an open field would doubtless catch insects too, but the number of insects passing that special spot is a restricted one compared with a place where they are for some reason or other concentrated. Such concentrations are not uncommon; the insects are, *e.g.,* more numerous along the border of a wood or field than in the middle of it. Most, if not all, flying insects have an instinctive fear of being blown away by the wind, and are therefore always trying to keep against it, thereby taking advantage of depressions and other irregularities of the earths surface, that will furnish them shelter or help them in advancing against the current. Stronger insects are not so dependent on shelter, but have nevertheless a special liking for streamlets, ravines, shores, wood-fringes, forest-roads, clearings, etc. where they patrol back and forth. Weak fliers very often prefer such openings to the dense wood. Such places are as a rule very good for traps, which must be expanded at right angles to the main direction, and preferably with the entrance away from the prevailing wind, so that insects working their way against the current may enter the trap". The collecting head or collector should always be in the sun, especially in the morning when most of the flight activity takes place. Protection from interference is first by finding secluded but still promising places; easiest on private property without free access. Sometimes this is impossible and protection is needed by *e.g.* barbed wire and attaching an information sheet for the public.

For an overview of preservatives, killing agents, frequency of change, quality of specimens, problems, precautions and treatment of the material, see Table 1. Ethanol may also act as an attractant for some groups (*e.g.*, insects associated with rotting organic tissue and their parasitoids). To avoid this 80% isopropanol may be used (Wilkening *et al.*, 1981), though unlike ethanol this will not preserve DNA and the condition of the specimens is only fair. A solution of 2.5% formalin should not be used; it is dangerous for the user, the specimens are irreversibly hardened and rendered useless for molecular studies. Cyanide (KCN or NaCN) is also dangerous and may cause extreme reddening of specimens.

Table 1 (next page). Overview of killing agents and preservatives and their effects on specimens.

Note: 96% Ethanol includes denatured ethanol B, and 70% ethanol includes suitably diluted IMS (= industrial methylated spirits). Dichlorvos (*e.g.*, Vapona strips) = 2.2-dichlorovinyl dimethyl phosphate; cyanide is KCN or NaCN encapsulated within plaster of Paris. Specimens killed by ethyl acetate vapour and air-dried specimens yield very degraded DNA (Dillon *et al*., 1996)

Killing agent/preservative	Availability	Maximum change over time	DNA preservation	Specimen condition	Problems / precautions	Minimum further treatment before	Optimal further treatment before
96% Ethanol	--	1 month	++	- (brittle)	higher evaporation and fire risk	rinse in acetone or 96% alcohol	AXA or CPD method
80% Ethanol or 80% Isopropanol	--	3 weeks	+	+ (fair)	id.	id.	id.
70% Ethanol	+	2 weeks	±	++ (good) (transfer to ethanol 80 or 96%)	swelling/softening if alcohol becomes too diluted	id.	id.
Ethylene glycol/ propylene glycol (+ alcohol) (antifreeze)	++	2 weeks	--	++	swelling/softening if glycol becomes too diluted	re-store in 80-96% ethanol/ rinse in acetone	id. (first re-store in 80-96% ethanol)
Satured salt solution (NaCl)	++	1 week	--	-- (poor)	damage by rinsing	id.	id.
Water + detergent ("soapy water")	++	2 days	--	--- (very poor)	disintegration of specimens	id.	id.
Dry with killing agent (cyanide or dichlorvos)	+	1 day	-	- (brittle + dirty)	high toxicity of agent to humans; add absorbent tissue	relax and clean before mounting	relax and clean before mounting
Dry without killing agent	++	few hours	-	- (brittle + dirty)	mutual physical damage (beetles!); add absorbent tissue	id.	id.

On average traps can be emptied daily (dry collecting), once per week (wet preservation, high season, tropics) or up to once per month (low season). This depends on the preservative used, the number of insects collected per day and the supposed use of the material. If 70% alcohol is used, the material will still be useful for molecular studies if the material is collected every week but it should be separated immediately and transferred to 80% to 96% alcohol. The material should be kept as cool and dark as possible; if the collecting bottle is subhyaline it may be covered by aluminium foil. In general the catch is first cleaned from large butterflies, moths and beetles (check for small insects clinging to them!), followed by pouring off the old preservative and replacing it by 70% or 80% alcohol. A fine sieve could be used to avoid losing minute specimens when the old preservative is poured off. A set of sieves of different mesh size can be used to sort the catch in several fractions, but this requires a lot of fluid and may cause damage to specimens. The sorting can be done by the unaided eye, with a head-lens or in small batches under a binocular microscope. The latter is the best option, but also the most time-consuming.

Safety

Fieldwork has its normal dangers for the researcher: in the tropics the chance of getting insect-borne diseases such as malaria and dengue can be lowered by using bed nets and prophylactic medicines against malaria. Impregnated bed nets are useful but may cause an allergic reaction. Legs and arms should be covered after 5 PM to lower the chance of contact by infected mosquitoes. Leeches are a nuisance but with the use of DEET on the shoes and eventually on leech-socks the problem is limited. The bleeding of the bites can be limited by using small pieces of tissue and the bites should be disinfected after bleeding has stopped. Both in temperate and tropical climates it is important to be aware of poisonous snakes. In case of allergic reaction to stings from aculeate Hymenoptera (*e.g.*, hornets and yellow jackets) an antidote should be taken in the field.

Preservatives used in the traps should be covered with a mesh or fine wire netting if there is a risk of its being drunk by mammals and birds; this is normally only a problem when there is an open reservoir below a flight interception trap. Some chemicals used in the traps, such as cyanide, dichlorvos and deltamethrin, are poisonous or can cause allergic reactions in humans and should be treated with care or avoided. During the processing of the material contact with xylene should be avoided and a fume-hood has to be used; if used outside the laboratory it should be done in a well ventilated room *e.g.*, by opening window(s) or in the open air.

In summary, a top 10 of the "does" and "don'ts" is given in Table 2.

Does	Don'ts
1. good position to block flyways or flight corridor	1. trap in shadow, *e.g.* collector of trap tight to a tree
2. good position for the collector: in the sun between 10 AM and 4 PM	2. leaving trap catches in sunlight after fetching
3. good position at border of habitat(s)	3. trap in habitat with a lot of butterflies (or use coarse mesh at the entrance of the collector)
4. perpendicular to a border when no flyway or flight corridor can be detected	4. bottle of collector filled up completely without free space above preservative
5. back of trap should be straight to guide the insects directly to the collector	5. placement near ant nest
6. monitor fabric near entrance of collector for holes and spider webs	6. use of 96% alcohol when the material has to be transported before sorting
7. clean inside of collector before use	7. trap well visible near places with many human visitors
8. inform local people about the traps and arrange protection with a fence of barbed wire or of chicken-wire netting	8. large traps in low vegetation because of unnecessary long distance to collector
9. reduce amount of alcohol or other preservative before transporting the catches	9. collector made of non-transparent material
10. refresh the alcohol or other preservative the same day after acquiring the catch	10. use of formalin

Table 2. Top 10 of good and of bad practices.

2. Traps with collector at top of trap, using positive phototropism

2.1. Introduction

The operation of the trap is based on the interception of the path of insects by means of a fabric or acrylic vertical or oblique barrier and subsequent positive phototropism. The intercepted insects are attracted by the sunlight to fly or walk to the top of the trap where the collector is situated. In principle, all flying insects are collected but groups with strong positive phototropism, such as most day-active Hymenoptera, Diptera and Lepidoptera, will be most abundant (Fig. 1A, B). Wingless insects and small flying insects may walk up the barrier ("diaphragm") in Malaise traps and the roof in Schacht traps to the collector, but the sampling is much less efficient than for actively flying insects. If small parasitoid Hymenoptera (mainly Platygastroidea, Chalcidoidea and Diapriidae) need to be collected, fine meshed material (mesh size 0.3-0.5 mm) should be used for construction. In most other cases a medium-sized (1.0-1.5 mm) mesh will be sufficient and may be more effective because of less interrupted air movement. The intercepted insects fly or walk to the collector, where they fall into a jar or bottle with a preservative.

2.2. Traps with a central diaphragm

2.2.1. Original unilateral and bilateral Malaise traps

Malaise traps are among the most important instruments for collecting day-flying (and to some degree also night-flying) species of Hymenoptera and Diptera. Other groups are also collected, but in general less efficiently (Figs 1 & 2). The trap is named after the Swedish Hymenopterist, insect and art collector Dr. René Edmond Malaise (1892-1978), who had the first versions made in Burma in 1934. He discovered the principle when he was camping in Sweden because of an opening in his tent where a considerable number of insects were gathered (Malaise, 1937). He proposed three types: a unilateral trap with lateral collector, a bilateral type with a lateral collector and one with a central collector. Even at that time he suggested the use of a framework to hang a bilateral trap in the canopy.

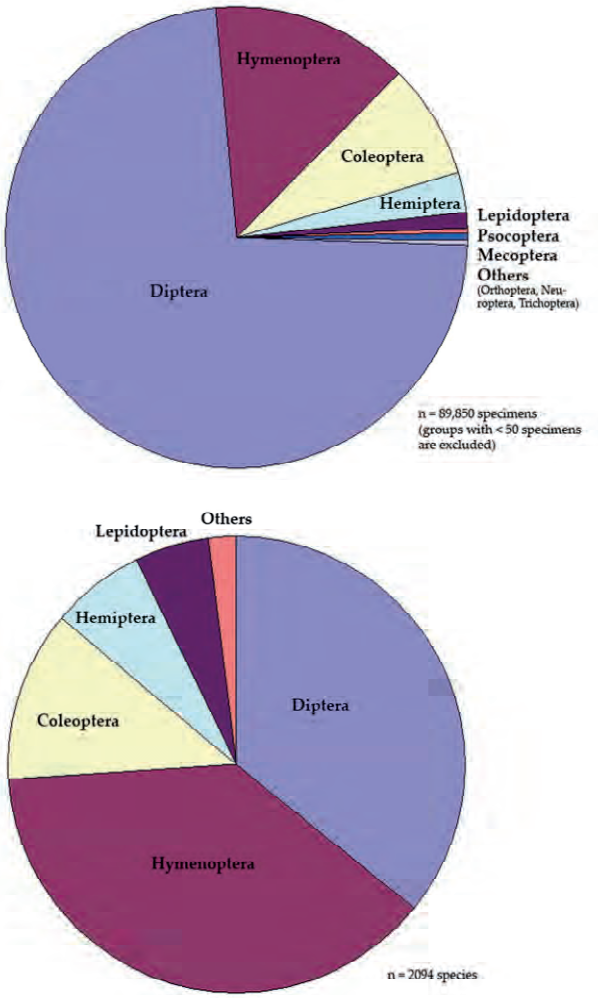


Fig. 1. Pie-diagrams of catches by a Malaise trap (Townes design) during 7 months (17.iii.-28. x.1990) in "De Brand", near Tilburg (the Netherlands; data from van Zuijlen *et al*., 1996).

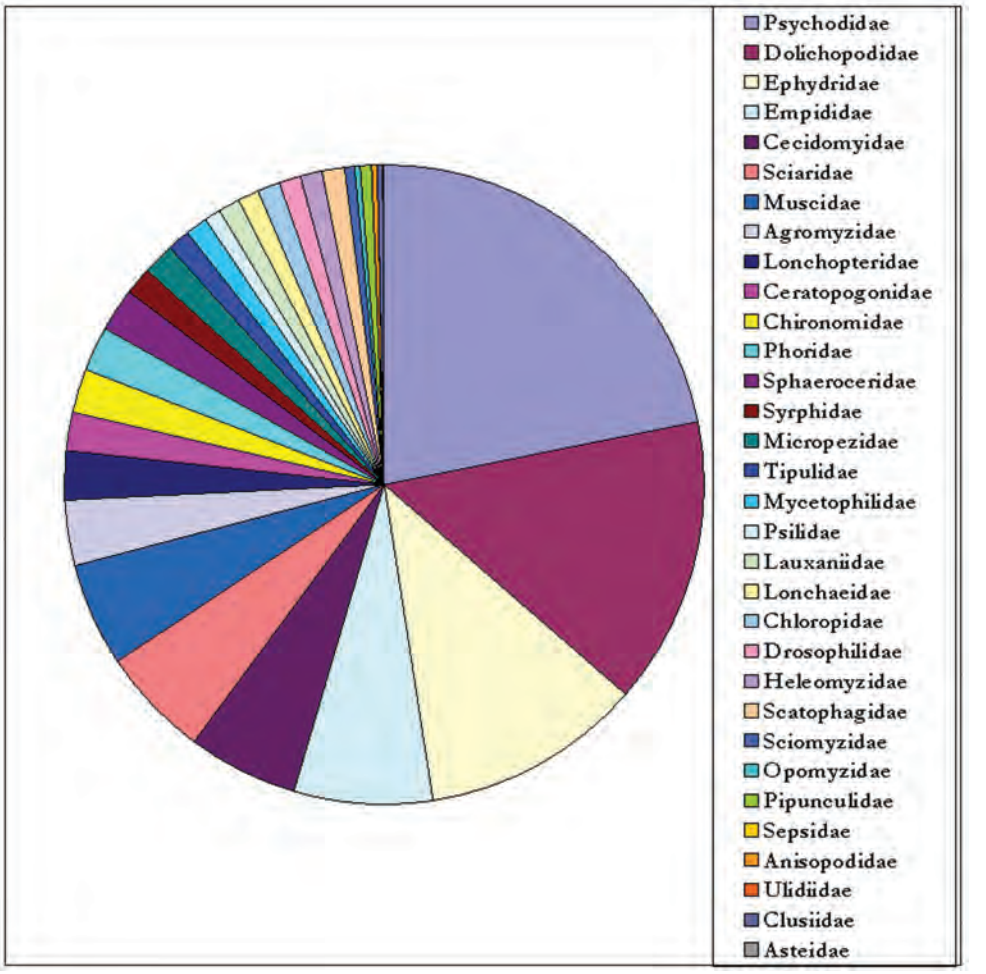


Fig. 2. Pie-diagram of Diptera catches by a Malaise trap in a very humid tropical biotope near a polluted river in SE Asia (P. Grootaert, unpublished).

The bilateral type with a lateral collector (Fig. 3) was used for the Townes design, but with the length of the diaphragm twice the depth of the lateral opening; the latter modification was also suggested by Malaise (1937).

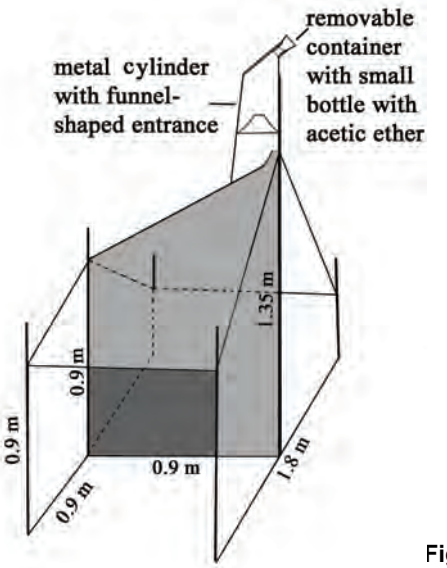


Fig. 3. Original design of bilateral Malaise trap.

2.2.2. Townes' redesign of the bilateral Malaise trap

A major break-through was the simplified design of Dr. Henry Keith Townes, Jr. (1913-1990) which he published in 1962. Townes type Malaise traps (Townes, 1962, 1972; Fig. 4) are the most commonly used design; they have a handy format and low weight, are open at two sides, with a diaphragm of about 1.6 m in the middle as barrier and with one lateral collector with a bottle at the summit. Either black with a white roof or completely black; the efficiency of having the trap white, black or bicoloured is a matter of continuing debate. The first author did not notice negative differences when using all-white traps compared with all-black traps; for some groups like sawflies and Syrphidae the catches seemed even higher than normal when completely white traps were used. A white object may better attract insects normally attracted to plants because it reflects all colours including yellow and green. The bilateral Townes design is vastly superior in collecting as compared with the "Cornell type" (Matthews & Matthews, 1983). The latter is a quadrilateral design with a central collector comparable to the SLAM (= Sea Land and Air Malaise trap) design (see Fig. 13).

Fig. 4. Townes design Malaise trap. (Photo by C. van Achterberg).

The collecting head or collector deserves special attention; the commercially available designs have a horizontal entrance and are degraded by UV light and/or are comparatively complicated and expensive. Hutcheson (1991) proposed a cheap, but not durable, alternative consisting of two polycarbonate bottles glued and taped together with the trap directly connected to the upper bottle. The first author designed in 1979 (Figs 5/6) a simple and durable collector with a 45º angled entrance made of PVC sewage pipe, at the top closed with a circular Perspex cutting and with an opening made opposite to the entrance and covered with a piece of Perspex (van Achterberg, 2009). It is almost indestructible, cheap and not degraded by UV light; the type recently made together with students at the Zhejiang University at Hangzhou is even cheaper to manufacture by using plastic drinks bottles (Fig. 7).

Fig. 5. Large grey PVC collector for Malaise trap (75 mm/45 degrees, 3.2 mm + insert) with 1 l bottle. (Photo by C. van Achterberg).

Fig. 6. Small grey PVC collector for Malaise trap (50 mm/45 degrees, 3.2 mm) with 0.2 l bottle. (Photo by C. van Achterberg).

Fig. 7. White UPVC collector for Malaise trap (Hangzhou type) (75 mm/45 degrees, 3.2 mm + insert) with 1 l bottle. (Photo by C. van Achterberg).

A half-height copy of the Townes design has been used successfully by the first author in relatively windy sites, when the vegetation is low and/or the trap needs to be inconspicuous to avoid theft. The half-height copies catch far fewer butterflies than the normal size and also have a smaller (two thirds the usual diameter) PVC collecting head, as designed by the first author in 1979 (Fig. 6). Large numbers of specimens may be collected and, if properly placed for several weeks or months in the right season, it collects a good sample of the fast and slow flying taxa present. Depending on the size of the trap, but normally from near-ground up to 0.8 m height, there is good sampling of the area.

Townes type traps can be used in nearly every habitat, even if no corridor for placement is available, *e.g.*, boreal tundra. Light-weight designs can be suspended in the canopy. The most commercially sold version of the Townes design has on average a total sampling surface (= sum of surface of both openings) of 3 m^2 (Matthews & Matthews, 1983), resulting in a sampling surface of 1.92 m^2 per m length of diaphragm. The designs are generally fairly weather resistant except under winter conditions with heavy (melting) snow loads on the roof of the trap. The traps are fairly portable and one person can set up a trap, but for large numbers of traps two people will perform much better.

Disadvantages are the cost (€ 100-400 per trap, depending on the design, place of manufacture and quality of the material), the visibility of the trap (they are fairly large objects difficult to hide from monkeys, humans, cattle, etc.), the time

needed to find promising places (preferably a corridor) and the total weight (normally including liquid preservative) if more than a few traps are used. Some of these disadvantages could be diminished by using thick thermo-sealed transparent Nylar film; not polyethylene plastic film, because that would deteriorate too fast in sunlight (Marston, 1965). The collector is made of a simple bag-shaped wire frame, covered with a bag and a second bag with alcohol is taped to it. Another approach is to use an insect bed net as a unilateral trap and add a plastic bag with some alcohol as collector at the top (Butler, 1965).

2.2.3. Malaise traps with two collectors

Gressitt & Gressitt (1962) published a greatly enlarged design; actually two Malaise traps joined at their rear parts, with two summits, each with a collector and a bottle. It results in a large trap (Fig. 8A) with the opening about 2.3 times longer than in the common Townes design: 6 m long in the commercially sold version (www.johnwhock.com). The trap has an opening at one side of 4.5 x 1.3 m, thus for both sides a total of 11.7 m^2 sampling surface, resulting in 2.6 m^2 sampling surface per m length of diaphragm. The migration trap is a modified Gressitt design: insects are separately collected per side to allow determination of the flight direction (Gressitt & Gressitt, 1962; Fig. 8B). The Gressitt design is frequently used for mosquito research. The design is made more complicated by having two collectors, and its large height (about 3 m) will negatively influence the catch of weakly flying and minute Hymenoptera. A recently developed smaller version (Fig. 8B) is lower and easier to place and a version with four collectors is being developed to determine four flight directions.

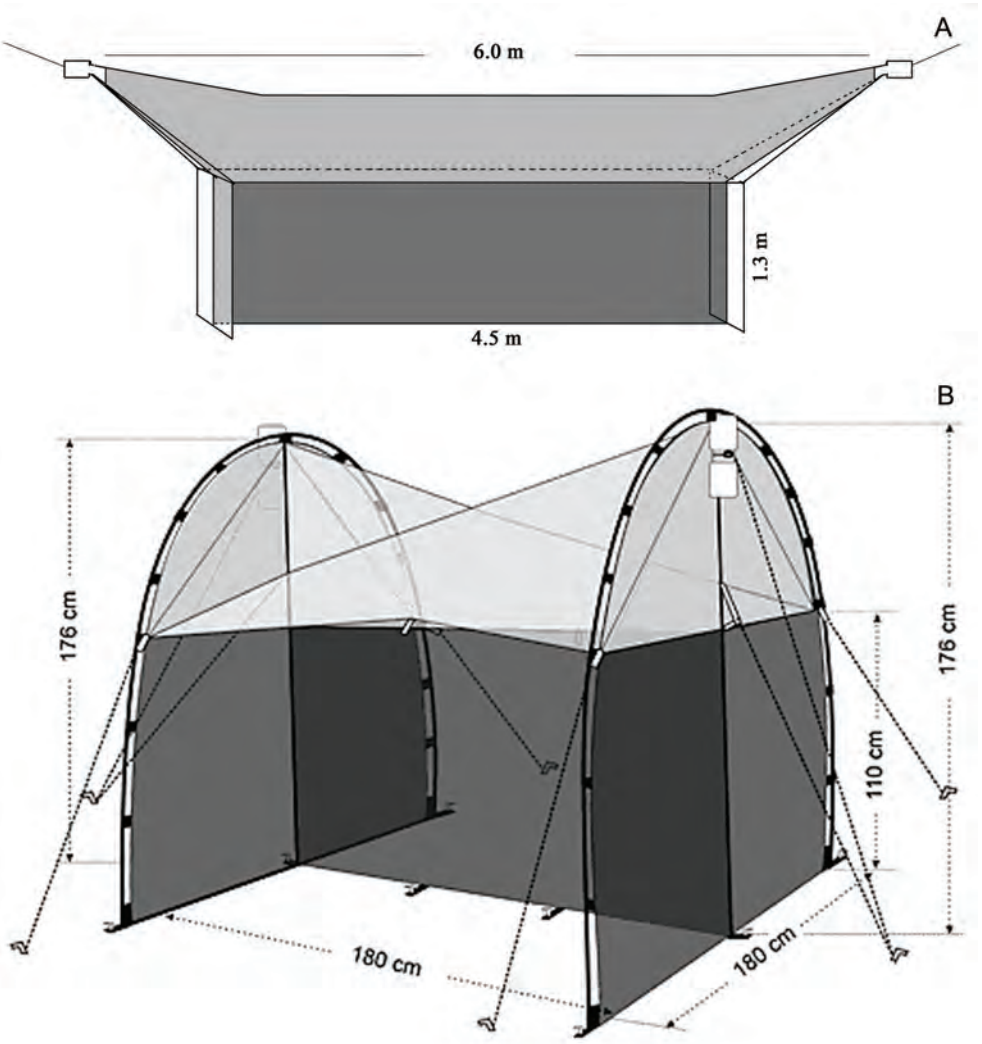


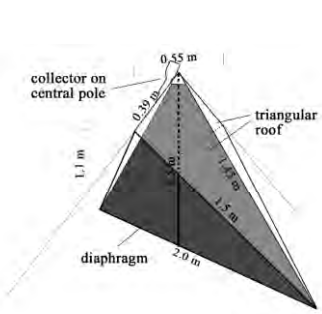
Fig. 8. A. Gressitt design of the Malaise trap; B. Scheme of small Gressitt design ("ez-migration trap") (from: http://bugdorm.megaview.com).

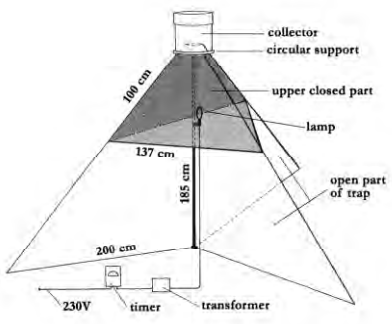
2.2.4. Malaise trap with triangular opening and a central collector

Three versions have been developed: the quadrilateral design (Cornell trap: Matthews & Matthews, 1983), the trilateral design combined with a light trap (Dufour, 1980; Fig. 9) and, recently, a light-weight bilateral design (Figs 10-12) by Mr. J. de Rond (Lelystad). The new bilateral design was aimed at collecting small parasitoid Hymenoptera (especially Bethylidae) in low open vegetation. The sampling surface is 1.1 m^2 per m length of the diaphragm, less than that of the Townes design, but the new design has a simpler construction, has a lower weight and should sample small walking parasitoids better. The first results are promising and the design is probably fairly weather-resistant. Its efficiency might

be improved by having the roof 30 cm wide at ground level (instead of a few centimetres in the prototype).

Fig. 9. Scheme of trilateral design combined with light trap (after Dufour, 1980).





Figs. 10-12. Bilateral Malaise trap with triangular opening and a central collector. Photos and sketch of design supplied by its designer, J. de Rond (Lelystad).

2.2.5. Freestanding, floating or hanging polyester fabric quadrilateral traps with a central collector

For use at a water surface or in the canopy the special SLAM (= Sea Land and Air Malaise) design has been developed (Fig. 13). It is freestanding (no supporting rods) and is easily erected by one person. It may be combined with a bottom collector(s) to become a hybrid between Type I and II flight interception traps (Fig. 14). The design with one bottom collector (Fig.15) is suitable for sampling different heights from ground level to the top of the canopy by attaching several free hanging traps to each other.

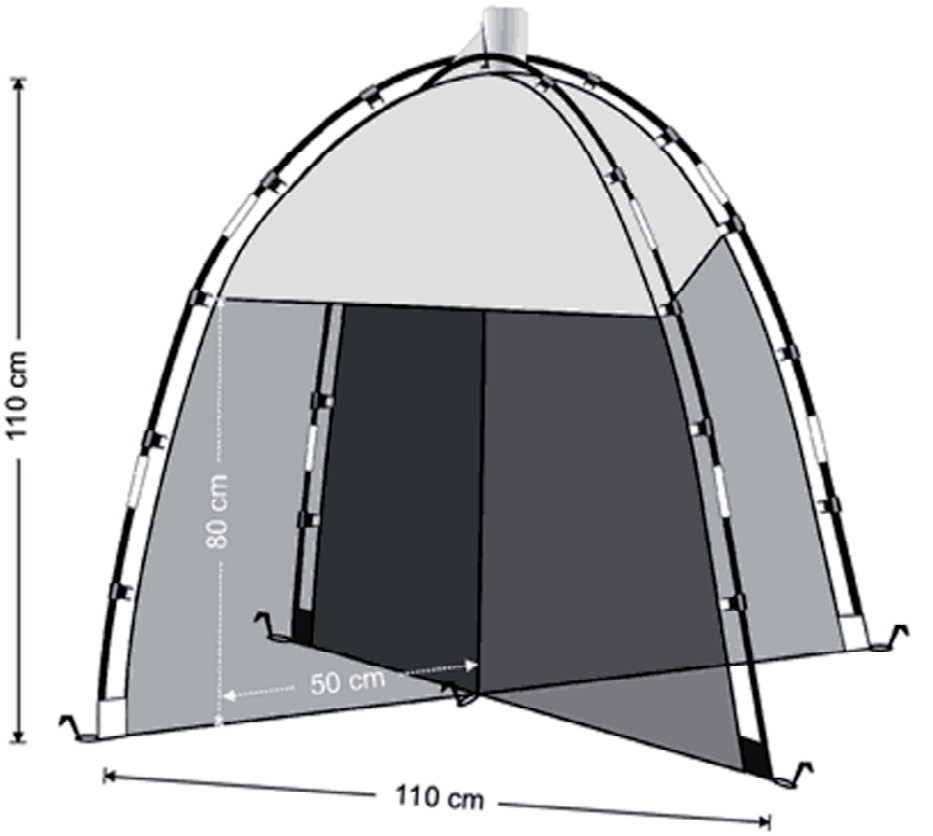


Fig. 13. Scheme of quadrilateral SLAM design (from: http://bugdorm.megaview.com).

Fig. 14. Hybrid SLAM design with collecting trays (from: http://bugdorm.megaview.com).

Fig. 15. SLAM design with a bottom collector (from: http://bugdorm.megaview.com).

2.2.6. Epsilon tsetse fly unilateral trap

This is a triangular fabric trap that attracts flies because it is contrastingly coloured. The oldest design was a box-type trap for collecting eye gnats and blow flies (Parman, 1931). It is easy to place and to remove for sampling tsetse fly populations (for details see www.nri.org and Fig. 16).

Fig. 16. Epsilon tsetse fly trap (from: www.nri.org).

2.2.7. Bilateral freestanding trap with rounded roof

Recently, a modified Malaise trap was developed with a rounded roof, no supporting rods and with a screen to prevent butterflies and large moths from entering the collector (Fig. 17). It is easily erected by one person and may be more weather-resistant than the Townes design. The sampling surface ratio of this design is 2.0 m^2 per m length of diaphragm, thus slightly improving the Townes design by about 5%. The incomplete diaphragm may have a negative influence on the efficiency of the trap, especially for larger arthropods. (for details see www.//http.bugdorm.megaview.com).

Fig. 17. Malaise trap with rounded roof design. (Photo by C. van Achterberg).

2.2.8. Redesigned bilateral Malaise trap

The sampling surface of the most frequently used type of Malaise trap, the Townes design (see above), is comparatively low. To enlarge the sampling surface (and probably its efficiency) the first author (van Achterberg, submitted) proposed an improved design to considerably enlarge the sampling surface without losing all the advantages and the simplicity of the Townes design. The redesign is based on four approaches. First is to direct the rear corners of the roof upwards (they are down in the Townes design), second to place the transverse sections more outwards (Figs 18 & 19), third to use a somewhat longer and higher diaphragm and finally to use the improved collector (see under Townes type).

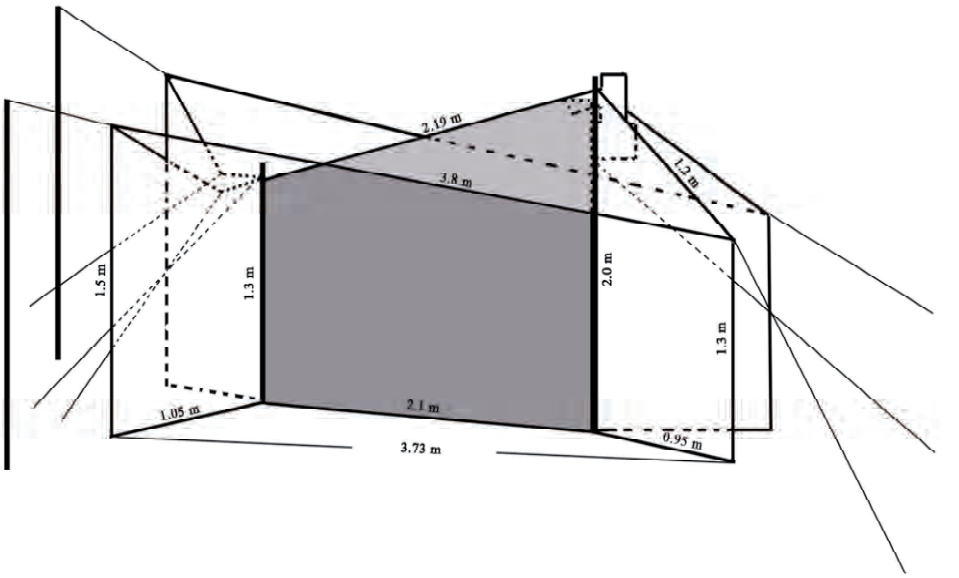


Fig. 18. Scheme of the redesigned Malaise trap.

Fig. 19. The first version of the redesigned Malaise trap. (Photo by C. van Achterberg).

The new design has a sampling surface ratio of 2.73 m^2 per m length of diaphragm, thus improving the Townes design by 42%. The ratio is similar to that of the Gressitt trap but the latter is twice as high and, therefore, less efficient if the height is taken into account. In addition, the Gressitt trap has two collecting heads and is heavier. The first impression of the catches by the new model is that the amount of specimens of some groups is about doubled, but the improvement differs per family. The trap has not been used for long enough to give comparative data yet. The new model will be commercially available in the near future; please contact the first author.

2.2.9. Freestanding quadrilateral Perspex trap with a central collector

Mr H.J. Vlug (Scherpenzeel) designed a small freestanding trap of two PMMA (= PolyMethylMethAcrylate, Plexiglas or Perspex) plates, triangular at the top, one indented at the base, the other at the top, and connected perpendicularly. On top of the plates there is a polyester fabric roof with a small central collector. This small trap is useful for collecting in low vegetation, but it is comparatively heavy and the construction of the collector is rather complicated.

2.3. Traps without a central diaphragm

2.3.1. Schacht trap

The Schacht trap (Schacht, 1988) was designed by Mr. Wolfgang Schacht (research associate at the Diptera section of the Zoologische Staatssammlung München). The trap is based on the idea that insects hitting an oblique surface will walk up the surface and, in the case of the trap, to the collecting bottle (Fig. 20). It is a rather new and little known trap, originally designed for collecting Diptera, but the Schacht trap may be recommended also for collecting Hymenoptera in addition to the use of Malaise traps. Although it is less effective, considering its size and the number of insects collected, it better collects small insects that tend to walk all the way up to the top, probably also because it works partly as an emergence trap. There is no diaphragm because it would deter insects; up to 80% of Hymenoptera flying into a Malaise trap may escape according to the late Dr. Townes (pers. comm.). The first results show that the Schacht trap is an excellent trap to sample a large area as a kind of emergence trap and it attracts (because it is a large white object) and intercepts a large variety of Diptera and Hymenoptera.

Fig. 20. Schacht trap (5 m long version). (Photo by C. van Achterberg).

2.3.2. Cheesecloth flight trap

This is a cage trap designed by Mr H.B. Leech (1955) for collecting Diptera and parasitoid Hymenoptera in large numbers. The trap has an equal-sided frame of 1.8 m covered with cheesecloth and with a door on the lower part of one side. The opening should be facing north or east; it traps insects in a way similar to, for instance, a garage with an open door facing north and a closed window at the other end. Herting (1969) used the same principle, but with dark textile for the sides and roof with a large opening at the back. The transparent front is against the wind; the trap needs to be checked several times per day and the numbers are rather low.

2.3.3. Manning trap

About thirty years ago the Manning trap was developed for collecting horse flies (Tabanidae). The dark (preferably black) central ball hangs free from an open box with a transparent cover with a central collector at the top (Fig. 21). The ball is warmed by the sun and is moved by the wind, mimicking a target for the flies. After discovering the lack of a suitable host they fly off to the sun and are intercepted by the upper part of the trap. Recently, the "LOER-2007" or "dazenval" (Dutch for horse fly trap) was designed by Mr. F. van Dungen (Heesch) for the same purpose. It has a massive black ball to attract the flies and is half covered by a white fabric hood; the flies are intercepted by the hood and

die in the central collector from heat on sunny days (Fig. 22). For collecting 200-400 horse flies per sunny day the ball should be far from ground level (the total trap height is about 3 m) and the trap should be placed near woodland edges and in the sun.

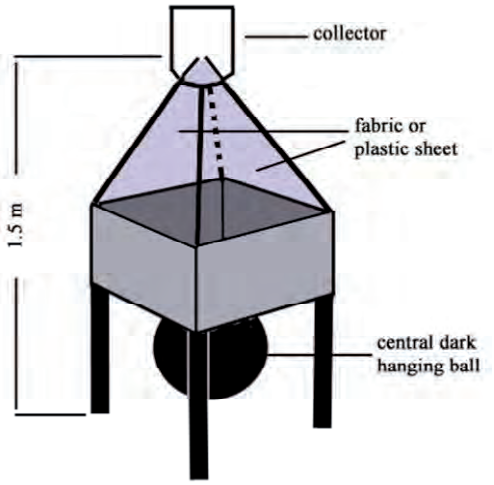


Fig. 21. Manning trap for collecting horse flies.

Fig. 22. Ball and hood (LOER-2007) trap for collecting horse flies. Left collector with dead flies (From: www.dazenval.nl).

3. Traps with collector at the bottom of the trap, using negative phototropism

3.1. Introduction

Many insects associated with the bottom layer of a micro-habitat fly just above ground level and fall to the ground when they collide with a vertical object. Flight interception traps with a bottom collector make use of this behaviour to trap insects, especially Coleoptera (Fig. 23). They are most effective at trapping relatively heavy, slow-flying insects such as beetles, cockroaches and crickets, groups that are hardly or not collected in Malaise traps.

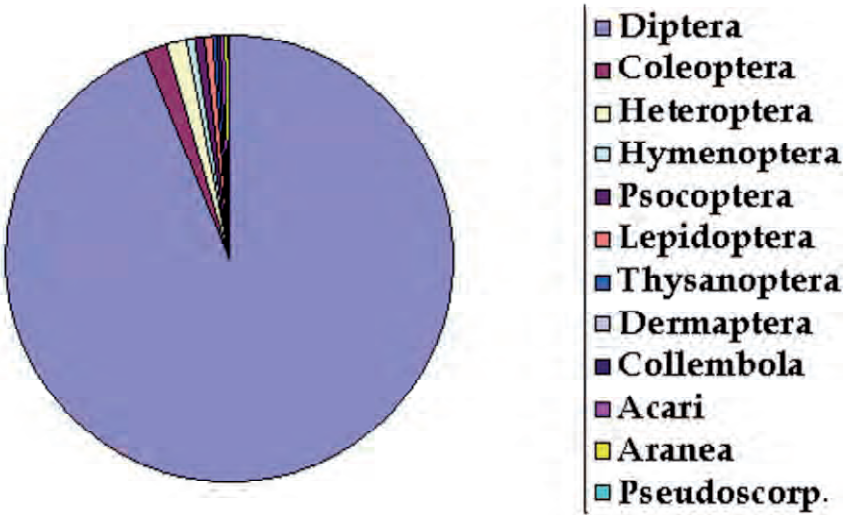


Fig. 23. Pie-diagram of catches by a window-pane interception trap in the temperate climate zone.

3.2. Transparent bilateral flight interception traps

A vertical screen of glass ("window flight trap" of Chapman & Kinghorn, 1955), Perspex or transparent plastic, such as PVC cling film, stretched between two stakes and a trough (or row of *e.g.*, ice cream containers) with preservative fluid (*e.g.*, water with propylene glycol and detergent) is arranged below its bottom edge (Figs 24-31). This is sometimes called a "window trap", but this name is applied to all kinds of unrelated traps, and therefore, the name "windowpane trap" is preferred for the framed types with glass, plastic or Perspex. A cover may be placed on top of the trap to avoid flooding by rain (Figs 25 & 26) and small holes may be made near the rim of the reservoir to allow overflow from rainfall without loss of trapped material. Nijholt & Chapman (1968) proposed a trap without fluid to collect living insects. The conical trough under the screen is open below and connected to a cylinder. The cylinder has a clear plastic bag or a removable glass jar at the end for collecting the live insects. Chapman & Kinghorn (1955) suggested the combination with a light source and the use of

transparent plastic screen without a frame. The presence or absence of a frame did not significantly influence the avoidance of the trap by Colorado beetles (Bouteau, 2000), though other tests have not been reported. A modified design has been used for sampling the forest canopy (Hill & Cermak, 1997; Fig. 26). If using collecting fluid is a problem the vertical screen can be made sticky and the insects adhere to the screen (sticky flight interception traps).

Fig. 24. Perspex bilateral window-pane interception trap. (Photo by P.S. van Wielink).

Fig. 25. Perspex bilateral window-pane interception trap with a plastic roof. (Photo by P.S. van Wielink).

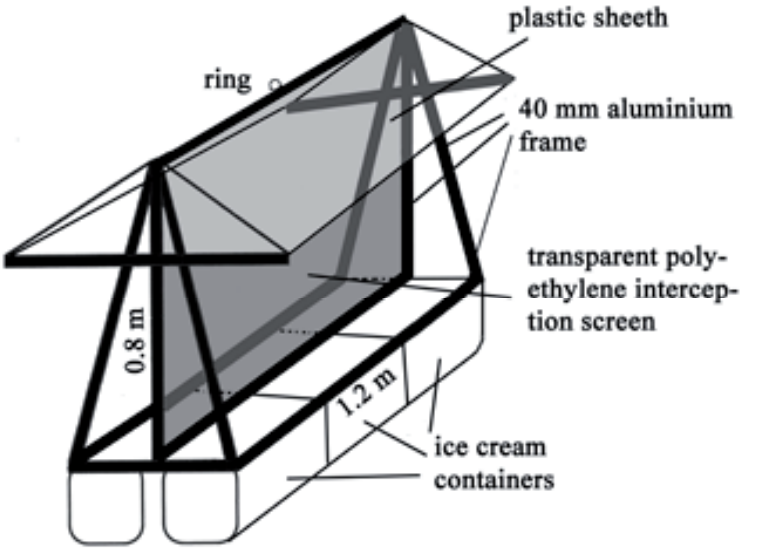


Fig. 26. Canopy flight interception trap with a polyethylene screen (after Hill & Cermak, 1997).

3.3. Transparent quadrilateral flight interception traps

Perspex window-pane interception traps with four collecting sides (= quadrilateral) are easier to place because of the 360 degrees collecting angle. This might either stand on four rods over an open reservoir with fluid (Fig. 27), or be constructed with an integral collector under it (Wilkening *et al.*, 1981). The latter version can be hung over a stack of wood or in a tree (Fig. 28) or combined with an upper collector (Wilkening trap; Fig. 29). Hines & Keikkenen (1977) and Furnes (1981) used a non-transparent cylinder for interception, *e.g.* one made of 33 cm diameter aluminium pizza plate.

Fig. 27. Perspex quadrilateral window-pane interception trap. (Photo by P. Grootaert).

Fig. 28. Suspended Perspex quadrilateral window-pane interception trap with a collecting bottle at the bottom. (Photo by B. Mériguet).

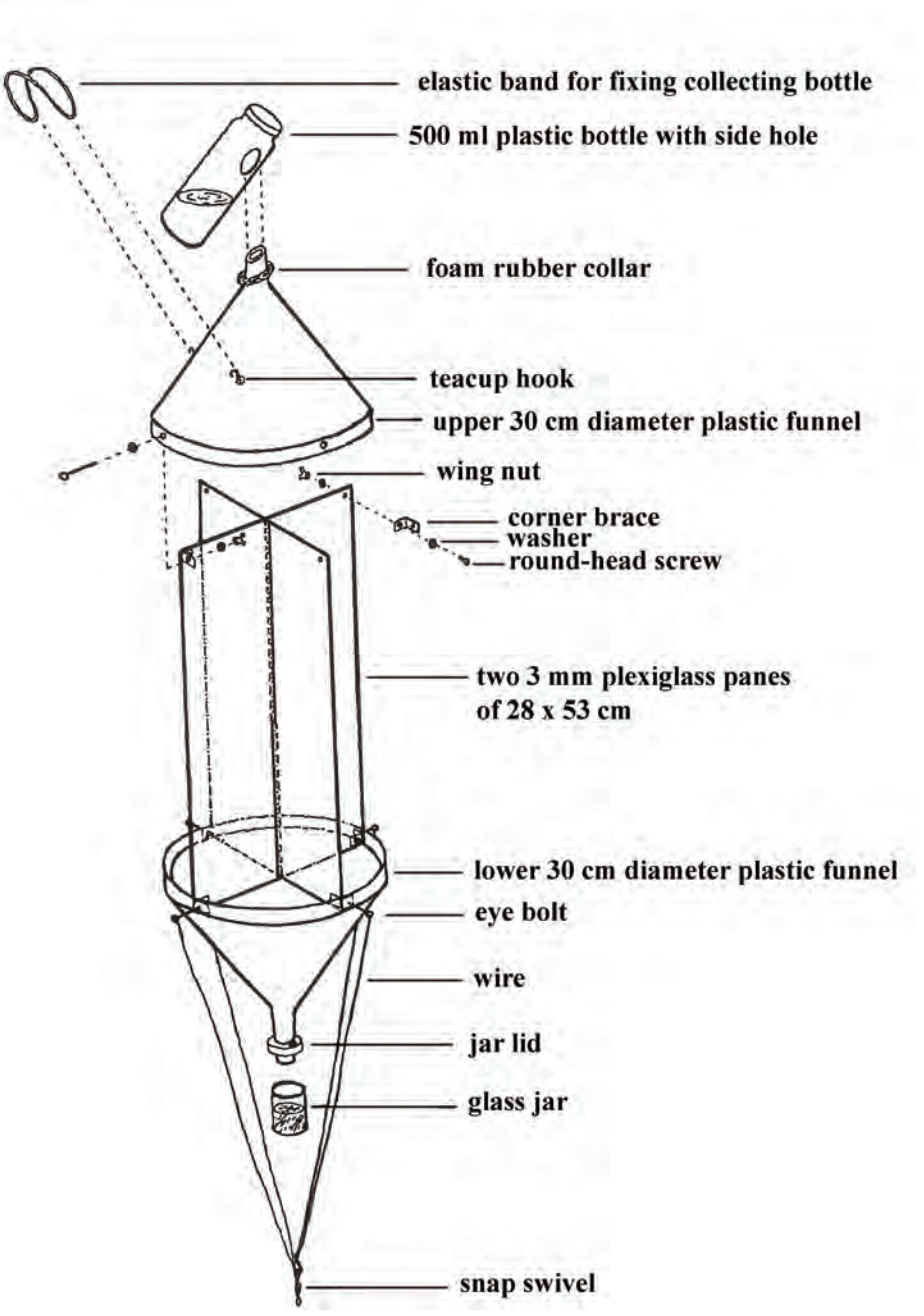


Fig. 29. Scheme of Wilkening trap (after Wilkening *et al.*, 1981).

3.4. Fabric screen interception traps

A vertical screen ("diaphragm") of fabric is stretched between two poles (Fig. 30). Trays, yellow pan traps or a plastic trough filled with water, a preservative and some detergent or with an antifreeze-alcohol solution are placed under the screen (Fig. 31). The disadvantages are the necessity of a flat horizontal area without protruding roots, stones, etc., the habit of some beetles to cling to the fabric with their claws and walk away, the need for transport of sufficient quantities of fluids, the risk of flooding by showers, the drinking of the fluids by vertebrate animals and the necessity to collect the captured insects at comparatively short intervals. Placing a plastic cover on top of the trap may avoid flooding by rain and using a bitter additive could avoid the drinking of the collecting fluid by animals. The EPPS biting fly trap (http://www.horselineproducts.com; Fig. 32) is designed for collecting flies, especially biting flies, near farms by providing a large, contrasting surface area and two semi-transparent areas (the deflectors). Many biting flies are attracted to large objects of contrasting colour (mimicking potential hosts like cattle, deer, and horses) and tend to circle around the host. Flies probably see the deflectors as open spaces, try to fly through, hit the deflectors, fall into the soapy water of the trays below and drown.

Fig. 30. Fabric interception trap (with separate trays). (From: http://www.inbio.ac.cr/papers/manual_coleoptera).

Fig. 31. Fabric interception trap with several small trays in a large tray. (From: http://mississippientomologicalmuseum.org.msstate.edu).

Fig. 32. EPPS fly trap using soapy water. (From: http://www.horselineproducts.com).

3.5. Trays below the diaphragm of a Malaise trap and use of insecticides

Yellow tray(s) with water, propylene glycol and a bit of detergent or a saturated salt solution are placed below the diaphragm of a Malaise trap (Figs 33 & 34; Robert, 1992). Insects (especially beetles) that bounce off will fall down into the trays with preservative. Masner & Goulet (1981) proposed the application of insecticide (pyrethroid: deltamethrin) to the diaphragm of the trap to make the collecting of small insects (especially Hymenoptera) more efficient. Altogether these measures will about double the collecting by a Malaise trap according to Campos *et al.* (2000). The disadvantages are the same as for fabric screen interception traps, but the results are much better.

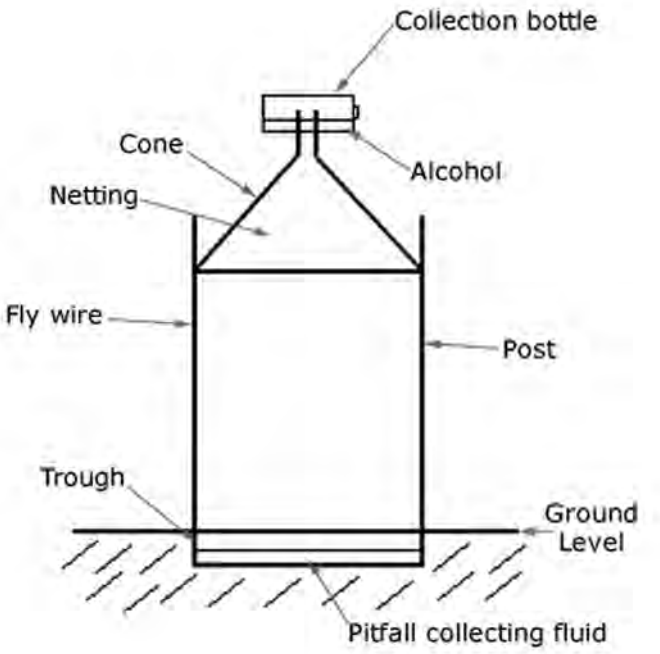


Fig. 33. Hybrid unilateral trap with rear diaphragm. (From: http://www.ento.csiro.au/education).

Fig. 34. Hybrid trap with the central diaphragm sprayed with insecticide and with a large yellow reservoir below it. T = top collector; B = bottom collector (From: Campos *et al.*, 2000)

4. Direct collecting

4.1. Suspended plastic bottles

A low-cost trap can be made from an array of 4 transparent, 2-liter polycarbonate beverage bottles suspended by their caps in a 2 x 2 array centred on the underside of a 20 x 30 cm piece of 1.3 cm thick exterior grade plywood. The plywood platform rests on four 2.5 m long metal rods; this conformation stabilizes it in windy conditions and protects it from rain (Fig. 35). The bottles each have a 17 cm wide and 13 cm high strip in its side removed to allow the entry of arthropods. When viewed from the side, the area of the opening in each bottle is 10.5 x 13 cm. The intact bottom of each bottle serves as a reservoir for about 200 ml of collecting fluid (Carrel, 2002). The preliminary results are similar to a glass or Perspex windowpane trap (*e.g.*, Dobony & Edwards' (2001) Perspex trap). The results might be improved for Hymenoptera and some other groups by painting the part of the bottle opposite to the opening yellow or white and the trap could be protected by wrapping chicken-wire netting around it.

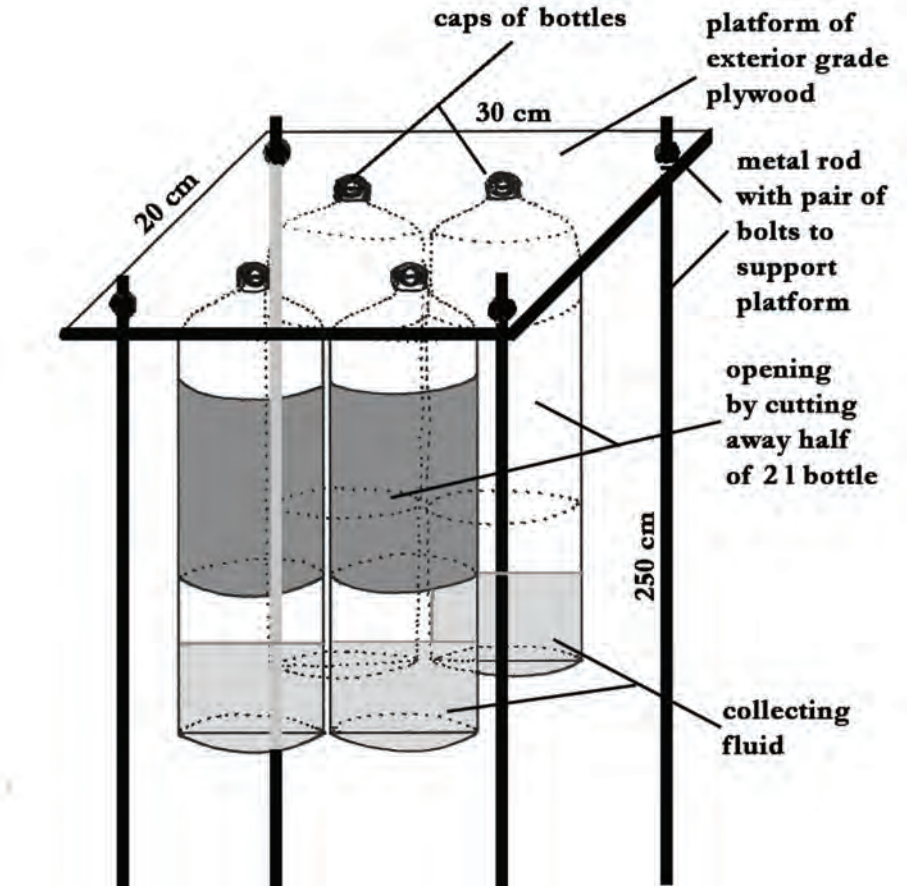


Fig. 35. Scheme of the plywood platform with four suspended transparent polycarbonate bottles.

4.2. Suspended sticky traps

These are usually yellow or blue plastic (polyethylene) or cardboard panels of 20 x 40 cm with a rain-resistant wet-sticky type of glue (*e.g.* Tangle) applied to both sides. The glue may be baited with pheromone to promote the collection of a certain group. The traps may be transparent, white or coloured: yellow for whiteflies, aphids, moths, leafhoppers and leaf mining Diptera and light blue for thrips. Also other groups will be collected by interception. The traps are widely available because they are used as part of integrated pest management programs in horticulture, being a non-toxic way to control and monitor insects. The glue does not dry out and the traps will last until the surface area is completely covered with insects (but they are of course prone to dust). Several traps are often suspended among vegetation, including the canopy. Recovering valuable specimens is problematic; the glue has to be resolved by warm kerosene, the specimens need extensive cleaning before preparation and fragile specimens will often be damaged. Although the low price of the traps and their easy use is a potential advantage, they are not usually a good method for specimen collection.

5. Acknowledgements

The first author thanks Prof. Dr. Xuexin Chen, Dr. Jiangli Tan and Mr. Shujun Wei (Hangzhou) for their help in assembling the new collector, Mr. Jeroen de Rond (Lelystad) for contributing illustrations and data on his recently developed trap, Mr. Paul van Wielink (Tilburg) for supplying illustrations of the windowpane traps and Mr. Theo Peeters (Tilburg) for providing information about the "dazenval", Mrs Josephine Cardale (Canberra) for kindly supplying details of CPD.

6. References

ACHTERBERG, C. VAN. 2009. Townes type Malaise traps can be improved? Some recent developments. *Entomologische Berichte Amsterdam* 69: 129-135.

BOUTEAU, G. 2000. Efficiency of flight interception traps for adult Colorado potato beetles (Coleoptera: Chrysomelidae). *Journal of Economic Entomology* 93: 630-635.

BUTLER, G.D. 1965. A modified Malaise insect trap. *Pan-Pacific Entomologist* 41: 51-53.

CAMPOS, W.G., PEREIRA, D.B.S & SCHOEREDER, J.H. 2000. Comparison of the efficiency of flight interception trap models for sampling Hymenoptera and other insects. *Anais da Sociedade Entomológica do Brasil* 29: 381-389.

CARREL, J.E. 2002. A novel aerial-interception trap for arthropod sampling. *Florida Entomologist* 85: 656-657.

CHAPMAN, J.A. & KINGHORN, J.M. 1955. Window flight traps for insects. *Canadian Entomologist* 87: 46-47.

CLEAVE, H.J. VAN & ROSS, J.A. 1947. A method for reclaiming dried zoological specimens. *Science* 105: 381.

DARLING, D.C. & PACKER, L. 1988. Effectiveness of Malaise traps in collecting Hymenoptera. The influence of trap design, mesh size and location. *Canadian Entomologist* 120: 787-796.

DILLON, N., AUSTIN, A.D. & BARTOWSKY, E. 1996. Comparison of preservation techniques for DNA extraction from hymenopterous insects. *Insect Molecular Biology* 5: 21-24.

DOBONY C.A. & EDWARDS, J.W. 2001. A new flight-interception trap for arthropod sampling. *Entomological News* 112: 217-220.

DUFOUR, C. 1980. Un nouveau piège lumineux pour la capture des Tipulidae et autres Diptères Nématocères: une tente "Malaise" lumineuse. *Mitteilungen der Schweizerischen Entomologischen Gesellschaft* 53: 313-320.

FURNES, M.M. 1981. An improved nonsticky trap for field testing of Scolytid pheromones. *Environmental Entomology* 10: 161-163.

GORDH, G. & HALL, J.C. 1979. A critical point drier used as a method of mounting insects from alcohol. *Entomological News* 90: 57-59.

GRESSITT, J.L. & GRESSITT, M.K. 1962. An improved Malaise trap. *Pacific Insects* 4: 87-90.

HERATY, J. & HAWKS, D. 1998. Hexamethyldisilazane a chemical alternative for drying insects. *Entomological News* 109: 369-374.

HERTING, B. 1969. Tent window traps used for collecting tachinids (Dipt.) at Délémont, Switzerland. *Technical Bulletin of Commonwealth Institute of Biological Control, Délémont* 12: 1-9.

HILL, C.J. & CERMAK, M. 1997. A new design and some preliminary results for a flight intercept trap to sample forest canopy arthropods. *Australian Journal of Entomology* 36: 51-55.

HINES, J.W. & HEIKKENEN, J.H. 1977. Beetles attracted to severed Virginia pine (*Pinus virginiana* Mill.). *Environmental Entomology* 6: 123-127.

HUTCHESON, J.A. 1991. Malaise trap collection jar: a cheap simple modification. *New Zealand Entomologist* 14: 48-49.

LEECH, H.B. 1955. Cheesecloth flight traps for insects. *Canadian Entomologist* 87: 200.

MALAISE, R. 1937. A new insect trap. *Entomologisk Tidskrift* 58: 148-160.

MARSTON, N. 1965. Recent modifications in the design of Malaise traps with a summary of the insects represented in collections. *Journal of the Kansas Entomological Society* 38: 154-162.

MASNER L. & GOULET, H. 1981. A new model of flight-interception trap for some hymenopterous insects. *Entomological News* 92: 199-201.

MATTHEWS, R.W. & MATTHEWS, J.R. 1983. Malaise traps: the Townes model catches more insects. *Contributions of the American Entomological Institute* 20: 428-432.

NIJHOLT, W.W. & CHAPMAN, J.A. 1968. A flight trap for collecting living insects. *Canadian Entomologist* 100: 1151-1153.

NOORT, S. VAN. 1995. A simple yet effective method for drying alcohol preserved specimens. *Chalcid Forum* 18: 3-4.

NOYES, J.S. 1982. Collecting and preserving chalcid wasps (Hymenoptera: Chalcidoidea). *Journal of Natural History* 16: 315-334.

PARMAN, D.C. 1931. Construction of the box-type trap for eye gnats and blow flies. *Bulletin of the United States Department of Agriculture* 299: 1-8.

ROBERT, J.C. 1992. Le piège Entomologique Composite (P.E.C.): une technique d'échantillonnage à large spectre de l'entomofaune terrestre circulante. *Mitteilungen der Schweizerischen Entomologischen Gesellschaft* 65: 395-411.

SCHACHT, W. 1988. Anleitung zum Bau einer Flugfalle mit diagonaler Fangfläche (Insecta). *Entomofauna* 9(15): 333-341.

TOWNES, H.K. 1962. Design for a Malaise trap. *Proceedings of the Entomological Society of Washington* 64: 253-262.

TOWNES, H.K. 1972. A light weight Malaise trap. *Entomological News* 83: 239-247.

TRUMEN, J.W. 1968. Acetone treatment for preservation of adult and larval mosquitoes. *Annals of the Entomological Society of America* 61: 779-780.

VOCKEROTH, J.R. 1966. A method of mounting insects from alcohol. *Canadian Entomologist* 98: 69-70.

WALPOLE, D.E., COETZEE, M. & LALKHAN, C.M. 1988. The use of acetone vapour for dehydration of insect specimens for scanning electron microscopy. *Journal of the Entomological Society of southern Africa* 51: 293-294.

WARE, A.B. & CROSS, R.H.M. 1989. Preparation of small delicate insects for scanning electron microscopy. *Proceeding of the Electron Microscopy Society of southern Africa* 19: 39-40.

WILKENING, A.J., FOLTZ, J.L., ATKINSON, T.H. & CONNOR, M.D. 1981. An omnidirectional flight trap for ascending and descending insects. *Canadian Entomologist* 113: 453-455.

ZUIJLEN, J.W. VAN, PEETERS, T.M.J, VAN WIELINK, P.S., VAN ECK A.P.W. & BOUVIER, E.H.M. (Eds) 1996. Brand-stof. Een inventarisatie van de entomofauna van het natuurreservaat “De Brand” in 1990. *Insektenwerkgroep KNNV-afdeling Tilburg*: 228 pp.

7. Appendix : Preparation of Hymenoptera and Diptera from alcohol

Most groups of unprepared Hymenoptera are usually stored in 70% alcohol. This is a safe method, but there are some hazards; dilution of alcohol (of whatever strength) in which specimens are stored should be avoided, otherwise a precipitate may form on the specimens. The specimens should be transferred to fresh 70% alcohol after being collected. Be sure that it is 70% or higher! Lower percentages often cause precipitation of dissolved fats, etc. and spoil the specimens. Never put vials containing specimens in alcohol in sunlight (UV-radiation, temperature!) and store samples in alcohol as cool as possible; to put them in the freezer is no problem. Dried out alcohol samples should not be discarded (van Cleave & Ross, 1947); with a 0.25-0.50% aqueous solution of a commercial grade of trisodium phosphate specimens are restored in a few hours (at 35° C in about one hour)!

The preparation of insects stored for a considerable time in 70% alcohol can be done well by three methods:

1. The most elaborate and most costly method is critical point drying (CPD; Gordh & Hall, 1979). The specimens are transferred to a small "basket" (a small numbered mesh container), which restricts the method only to small specimens. The results for *e.g.*, Eulophidae (Hymenoptera) are much better than air drying as the heads do not collapse. Freeze-drying is a similar method.

2. The Alcohol/Xylene-Amyl acetate-method (AXA); a less expensive and less time-consuming method than critical point or freeze-drying and the results are usually comparable. It is also suitable for large Hymenoptera and large quantities can be treated at once. It is based on the alcohol-ethyl acetate method used for the preparation of Syrphidae in the Canadian National Collection of Insects at Ottawa (Vockeroth, 1966). The ethyl acetate was replaced by amyl acetate by the late Dr. W.R.M. Mason (working at the same institute) for the preparation of Braconidae from 70% alcohol. The first author successfully used the modified version explained below during over 30 years for Braconidae and other Hymenoptera in the collection of the National Museum of Natural History (Naturalis) at Leiden.

The alcohol is poured off (carefully, to avoid loosing specimens) and the vial is filled with a mixture of 40% xylene and 60% alcohol made out of a concentration of 96% alcohol. After 1-3 days this mixture is poured off again and replaced by amyl acetate; do not use any kind of (plastic) vials that are susceptible to amyl acetate and avoid inhalation of the chemicals or contact with the skin. The insects can be prepared after 1 day (or longer) in the amyl acetate. With forceps the specimens are taken from the fluid and with the wings stretched out laid on any kind of slowly absorbing paper (*e.g.*, 180-250 grivorite paper). If the wings are not well stretched out, the procedure should be repeated or a drop of fluid is added with the tip of the forceps. After about 15 minutes the specimens are ready to be pinned or glued. Pinning should be done not later than 25 minutes after taking out of the amyl acetate to avoid losing legs or its head during pinning. An alternative is to put a limited number of specimens in a thin layer of amyl acetate and let it evaporate.

3. Heat-assisted air-drying from acetone (Trumen, 1968; Walpole *et al.*, 1988) is an easy and fast method for specimens preserved in alcohol for less than one year. The specimens may be removed from the 70% alcohol and kept for a few hours in water, followed by a few hours in acetone. If the specimens are cleaned before by rinsing them in 70 or 80% alcohol the results are generally slightly less than of the AXA method or CPD. However, according to Ware & Cross (1989) and van Noort (1995) the results are the same for some groups of Chalcidoidea. The direct slow drying of the alcohol (Noyes, 1982) gives much worse results, especially the wing venation is often less visible because of distortion of the wings. The latter method lowers considerably the quality of the material of relatively weakly sclerotised, delicate or small specimens (like Braconidae, Chalcidoidea and Diptera) and should be avoided unless the specimen is collected within a few hours. However, for many relatively robust and large Ichneumonidae, rinsing in 96% alcohol and drying onto absorbent tissue (which will often enable the wings to dry flat) can be the most practical way to achieve fairly good and consistent results. Some specialists advocate the use of HMDS (hexamethyldisilazane) for insects (*e.g.*, Heraty & Hawks, 1998), but the chemical is expensive (about € 900 per kg plus shipping costs), and in some trials with Braconidae and Chalcidoidea the results were less good than those obtained with the CPD, AXA or acetone methods. In addition, HMDS has an unpleasant smell, is highly flammable and has a strong corrosive effect on eyes and to a lesser degree on skin and mucous membranes.

8. Glossary

AXA method: the use of xylene and amyl acetate to prepare material from alcohol.

Bilateral trap: trap with two open sides or 180º collecting angle.

Central collector: collecting device situated at centre of the trap.

Cornell type Malaise trap: small quadrilateral Malaise trap.

CPD: critical point drying method.

DEET: an insect repellent: N,N-diethyl-meta-toluamide.

HMDS: hexamethyldisilazane.

Lateral collector: collecting device situated at one of the sides of the trap.

PMMA: Perspex or polymethylmethacrylate.

PVC: polyvinylchloride or polychlooretheen (PCE).

Quadrilateral trap: trap with four open sides or 360º collecting angle.

SLAM: Sea Land & Air Malaise trap design.

Unilateral trap: trap with one open side or 90º collecting angle.

UPVC: unplasticised polyvinylchloride.

Chapter 18

Preserving and Specimen Handling: Insects and other Invertebrates

by

Lars Krogmann
Staatliches Museum für Naturkunde Stuttgart (SMNS)
Rosenstein 1, 70191 Stuttgart, Germany
Email: krogmann.smns@naturkundemuseum-bw.de

Joachim Holstein
Staatliches Museum für Naturkunde Stuttgart (SMNS)
Rosenstein 1, 70191 Stuttgart, Germany
Email: holstein.smns@naturkundemuseum-bw.de

Abstract

Up-to-date field techniques for preserving and handling invertebrate specimens with special emphasis on insects are summarized. Different preservation techniques for specimens sampled for molecular and morphological analyses as well as for natural history collections are presented and hints for scientific labelling of specimens in the field are given. Fluid fixation of molluscs, annelids, nematodes, and plathelmints are briefly discussed. Fluid or dry preservation of insects and other arthropods depends on the purpose of the field work and on the taxonomic groups. The most commonly used killing agents, fixation fluids and sample containers are discussed. Direct pinning and card mounting of insects in the field is explained and hints for transport of specimens are given. The recipes of frequently used reagents and solutions are listed in an appendix.

Keywords: specimen sampling, killing, labelling, transport, fluid preservation, dry preservation, direct pinning, card mounting

1. Introduction

This chapter will discuss the steps following immediately after the collecting event. The first consideration is what is the purpose of the fieldwork, *e.g.,* what is supposed to be done with the collected invertebrate specimens. If the specimens shall not be kept alive for experimental studies or for rearing larvae to adults (this will not be dealt with in this chapter) the first logical step is the killing or in some cases the narcotisation of the specimens. If traps like Malaise traps, flight interception traps, yellow pan traps or pit fall traps have been used this step can be skipped in most cases as the specimens are normally killed by a fluid with which the collecting containers in the traps were filled. When the specimens have been collected alive it must be considered if the specimens will be used as a whole, *i.e.* as voucher specimens in natural history museums or if they shall wholly or partly be used for molecular or morphological investigation.

2. Specimen sampling and labelling

2.1. Specimen sampling for molecular analyses

Molecular analyses, such as DNA sequencing, require particular considerations that preserve DNA and are only briefly considered here. Usually the best option to preserve the DNA for long time storage is to transfer the specimens to high percentage ethanol (95-99%) which serves as both, as killing and fixation agent. A second option is to keep the specimens alive during transport and have them fresh frozen in the laboratory. This allows storing suitable DNA for decades. The biggest enemy of DNA is humidity so long time storage in low percentage ethanol (70%) should be avoided as well as leaving the specimens in moist atmosphere. Specimens preserved in ethanol should be put in dark and cool conditions as soon as possible and not left out in daylight during fieldwork (or in the laboratory!). This applies to all invertebrate specimens sampled in ethanol whether they are to be used for DNA studies or not. If only low percentage ethanol is available it may a better option to kill the specimens with a killing agent, let them dry quickly and store them (or just selected body parts of them) in the freezer and/or high percentage ethanol in the laboratory. Even if the specimens are stored in dry collections they usually allow extracting suitable DNA for ten or more years (in some cases even hundreds of years) but it seems that the success rate decreases significantly over time.

2.2. Specimen sampling for morphological analyses

If specimens shall be used for morphological investigation (*e.g.*, anatomical dissections, thin-sectioning) it is generally most appropriate to kill and store them directly in a fixation fluid. The fluid normally depends on the taxonomic group and/or the morphological analyses. Specimens that will be used for histological work can be preserved in a number of different fluids that often contain formalin. Fluids like Kahle's or Bouin's solution (Appendix 1) are the best choice for insect larvae as they fix tissues. Kahle's solution also prevents larvae from discoloration while Bouin's solution may change the colour of larvae to light yellow. Before formalin is used it should be considered that it contains

formaldehyde which cross-links proteins and makes tissue samples unusable for DNA extraction.

2.3. Specimen sampling for natural history collections

In the majority of cases specimens are collected to be stored in natural history collections for documentation and research. Even though this does not preclude that parts of the specimens may still be used in future molecular or morphological studies, the primary purpose of the fieldwork is to yield specimens that should be preserved and stored as a whole. The question that arises is if the specimens are to be stored in a dry or a wet collection (*i.e.* usually an ethanol collection). In most cases specimens that are to be stored in an ethanol collection will already be killed and fixated in ethanol directly in the field (70-80% is the standard ethanol concentration). It can be suitable to add a small amount of glycerol to the ethanol, which makes the specimens less stiff. Also glycerol does not evaporate which can be an advantage when containers do not close hermetically. However, use of glycerol should be avoided for small winged insects, such as Micro-Hymenoptera as it complicates the subsequent dry-mounting of these specimens. Glycerol softens the wings in small insect specimens excessively, so that they will not stay flat when specimens are air or critical point dried and card-mounted. Only for small winged insects that need to be slide-mounted (*e.g.* Thysanoptera), glycerol-ethanol solutions are a good option. Ethanol vials should be completely filled which makes specimens less prone to damage during transport. Even small air bubbles that slosh around in the vials can cause damage to very fragile specimens, so special care should be taken to minimize these risks in the field. It should be considered that glass vials that are completely filled with ethanol may crack or even explode in the hold of an aeroplane. With plastic vials these problems can be overcome but it is still useful to seal the screw-cap of the vial with stripes of Parafilm® as it may become loose or undone during transit. An authorization is needed to transport ethanol in an aeroplane and therefore dry storage of specimens (see 3.3.4) during transit is more advisable. Specimens that are to be deposited in a dry collection are normally killed by a gaseous killing agent and stored dry before they are further processed (*e.g.,* pinned and mounted). Keeping and storing specimens dry in the field usually requires more care from the collector as specimens are more fragile and prone to damage compared to specimens preserved and transported in a fluid fixation agent. This is even more severe when specimens are completely dried, which can occur within a few hours on a hot and dry collecting day. Especially dry insects are very delicate and care must be taken to prevent specimens from loosing legs, heads or antennae during transport. Many collectors therefore transport the specimens in a moist atmosphere, which can be a plastic box that is laid out with wet tissue. A few drops of thymol-camphor solution (Appendix 1) or a few crumbs of crystalline thymol should be added to the tissue to prevent the specimens from moulding. If smaller specimens numbers are collected it may also be appropriate to pin the specimens directly after collecting (*e.g.,* in the field or immediately after in the hotel or field station) which secures specimens and facilitates subsequent preparations. Special transport boxes can be obtained from entomological suppliers. Also in these dry boxes it is appropriate to add thymol as larger specimens that cannot dry fast may get mouldy.

2.4. Labelling

Even experienced biologists tend to inappropriately label specimens so this step needs special attention as biological specimens loose their significance for research and documentation if they are not or insufficiently labelled. Labelling should be done in the field, directly after collecting the specimens or after emptying the traps. It can be convenient to prepare the labels in advance and already print parts of the information (*e.g.*, parts of the locality data, name of collector) beforehand and just add the specific data (*e.g.*, date and altitude) in the field. External labelling of tubes or transport boxes can be useful but does not replace a proper labelling of the individual specimens or samples inside the respective container. The most widespread mistake during fieldwork is to just add numbers to the specimens and to list the collection data on separate sheets. Even though the collector has strong intentions to properly label his samples "some when" after the fieldwork there is always a high risk that this will never happen and that the collected specimens will loose their scientific value.

It is crucial that specimens are labelled with all necessary collection data:

- **Locality** (Country, Province, nearest City, Region)
- **Name of project** (if available)
- **GPS data** (if available)
- **Altitude**
- **Collecting method**
- **Date of collection**
- **Name of collector**

Further data (*e.g.,* habitat type, host plant, weather, and temperature) should also be added on (an) additional label(s). The golden rules (Table 1) should be followed to minimize the risks of mixing samples or loosing locality information.

Labels for dry specimens should be written with water proof pens or pencils. Labels for ethanol vials should preferably be written with ethanol-proof ink, *e.g.*, Micron archival ink pens (SAKURA corp.) or alternatively with a pencil. Laser printed labels will not last in ethanol and should not be used. Wet preserved samples should be generally labelled on tight paper which is not negatively affected by the fixation agent. Handwritten labels can later be replaced by proper type-written labels in the laboratory but care needs to be taken that spelling mistakes are avoided. Long-term storage and viability of ink on collection labels is a big challenge for curators of natural history collections and cannot be addressed in this field manual. However, every collector should make sure (prior to collecting) that long term storage of his natural history specimens and the necessary curatorial care can be guaranteed by the respective institute.

Rule 1	Always label the specimens and add the collection information to the specimens immediately (*i.e.*, directly in the field).
Rule 2	Preferably every specimen gets an individual label, but if this cannot be achieved due to high specimen numbers at least every sample gets an individual label.
Rule 3	A sample contains only specimens which have identical collecting data and which are clearly separated in an individual container from the other samples.
Rule 4	If specimens are pre-sorted into smaller samples every sample needs to get a proper label.
Rule 5	Labels are always placed inside the vials, labelling the vials just from the outside is insufficient.
Rule 6	Numbering of samples does not replace locality labels and may later result in confusion and loss of information.

Table 1. The six golden rules for labelling scientific specimens in the field.

3. Invertebrate taxa

The right treatment of collected invertebrate specimens is not only dependent on the purpose of the collecting (see 2.1-2.3) but also on the invertebrate taxon and its life history stage. Soft bodied invertebrates generally require fixation as they suffer from shrinkage if air-dried while hard bodied, sclerotized invertebrates can often be air-dried and may even be damaged if put in ethanol. However, there are many exemptions from this rule and many taxa require a special treatment which made it necessary to devote a separate chapter to the different terrestrial and limnic invertebrates that can be the subject of fieldwork.

3.1. Molluscs (Mollusca)

If the soft parts of the animals shall be preserved as well as the shell (if present), it is necessary to narcotize the specimens prior to the killing. This ensures that the organisms are expanded and fully display their characteristic features. For this, terrestrial gastropods are best placed in a jar of water. The animals will die in a relaxed position (outside the shell if it is present) within 1 or 2 days (Sturm *et al.*, 2006). Afterwards the specimens should be transferred into a preservative, which can be 80% ethanol, a mixture of ethanol (80%), water (15%) and glycerol (5%), or formalin. Several different preservation methods have been described for molluscs (see Piechocki & Händel, 1996; Sturm *et al.*, 2006) but not all of them are practicable for field trips.

Molluscs, which are anticipated to be included in DNA studies, should be transferred immediately after collecting into 95-99% ethanol.

3.2. Round worms (Nematoda), flat worms (Plathelminthes), and segmented worms (Annelida)

Nematodes are usually killed and preserved in the laboratory after they have been extracted from plant or animal tissue or from soil samples. Due to their small body size, nematodes are always handled in fluid medium under a dissection microscope. General techniques for handling, killing and preserving nematodes are summarized in Kleynhans (1999).

Flat worms are a diverse group of organisms from which only the Turbellaria contain non-parasitic groups. The parasitic groups are not included here but their preservation is discussed in Piechocki & Händel (1996). Aquatic and terrestrial Turbellaria are best preserved in FAA (Appendix 1). Alternatively, formalin (5%) can be used as a suitable fixation agent. The problems of specimen contracting can be overcome by a variety of techniques which are discussed in Knudsen (1972) and Piechocki & Händel (1996). Some of these techniques require the use of mercuric chlorides which we would not recommend (especially not in the field) due to its toxic nature. Final storage of the flatworms should be in formalin (5%) or in ethanol (70-80%).

From the segmented worms (Annelida), only free living earthworms (Oligochaeta: Lumbricidae) and leeches (Hirudinea) are dealt with here. Oligochaete worms shall not be placed immediately in ethanol (unless they are to be used for molecular study) as they shrink. The specimens are washed in a shallow dish and killed in a weak formalin solution (1-2%). It is important to slew the specimens with forceps constantly in the formalin solution which limits the number of specimens that can be dealt with to about five specimens per treatment. After the oligochaete worms got immobilized they are stretched outside the solution. The specimens are then placed on blotting paper and permanently wetted with formalin solution. Alternatively, specimens can be covered by cellulose which has been imbued with formalin. After the specimens hardened (after 30-40 minutes) they need to be transferred into glass vials, which should be long enough to house the specimens. The vials can be either filled with formalin solution (5%) or ethanol (70-80%). Leaches are narcotized in 5-15% ethanol until they do not show any reactions anymore. This may take ½ to six hours depending on the size and physiological condition of the specimen. Fixation occurs in formalin solution (1:4), ethanol (70%) or formol-alcohol (Appendix 1).

3.3. Arthropods (Arthropoda)

Arthropods are the most diverse group of terrestrial organisms and their overall abundance and diversity makes them an important target group for fieldwork. Soft bodied arthropods are best transferred by spring steel forceps which allows safe handling without damaging the specimens (Fig. 1). Small, hard bodied arthropods are best handled or divided into smaller samples with the help of an aspirator (Fig. 1). In the following we will discuss different preservation methods for arthropods, which are best practicable in the field.

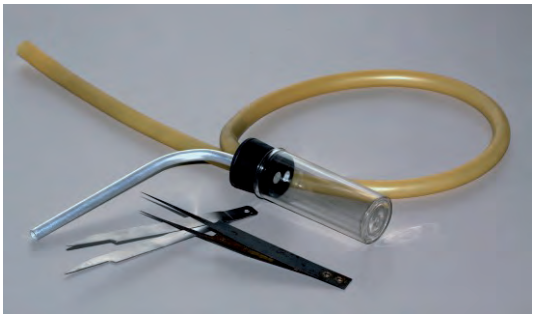


Fig. 1. Handling arthropods in the field. Delicate, soft bodied specimens can be handled with spring steel forceps. Minute, hard bodied specimens are best transferred by an aspirator which can be obtained from entomological suppliers. (Photo by authors).

3.3.1. Fluid preservation of arthropods

There can be a difference between the collecting and fixation fluids which are used for fieldwork and fluids which are used for permanent preservation in natural history collections. Here we only discuss those methods which are used during fieldwork, *i.e*. which concern the collecting and short-term storage during transport. In most cases specimens are directly killed and preserved in the same fixation agent but sometimes the killing agent can differ from the fixation agent. For example, it can be more suitable to collect (and kill) arthropods in water or salt water (*e.g.*, in a pan trap) as ethanol or other fixation agents may act as repellent or attractant thus artificially altering the species composition and diversity of the samples. However the time specimens are kept in non-fixation agents must be held to a minimum as specimens will start decaying within 1-2 days (depending on the temperature).

Standard fixation fluids for arthropods are:

- 70 -80% ethanol (higher alcohol concentration should only be used when specimens are to be included in molecular investigations). This is by far the most common fixation agent and suitable for the vast majority of arthropods.

- 2% acetic acid (like the concentration of vinegar), also feasible for permanent preservation. It is normally used for well sclerotized taxa like Coleoptera, Heteroptera or ants, which shall be dry- mounted in the laboratory. The specimens stay soft and elastic and normally do not need to be relaxed prior to dry-mounting. However, acetic acid is not feasible for most Arachnida and Crustacea because the specimens become too soft and Crustacea will even loose their integumental calcium deposits.

- Acetic acid-glycerol-alcohol solution (AGA) is suitable for small wingless arthropods such as mites (Acari) and for small winged forms like thrips (Thysanoptera). AGA is not suitable for winged forms that are intended to be dry-mounted.

- Lactic alcohol is suitable for aphids (Aphidoidea) and scale insects (Coccoidea).

- Saturated picric acid solution (odourless, only used as fixation liquid, sample has to be transferred into ethanol afterwards). A negative side effect is that specimens fade into yellow according to the luminous yellow colour of the picric acid.

The standard preservation fluid for short and long term storage of arthropods is 70 -80% ethanol. There are a number of different vials available from which those with a screw top should be preferred. Glass vials (Fig. 2) are commonly used but during field work they always bear the risk of being broken, which may lead the specimens to be lost and the collector to be injured by scattering glass pieces. The best option for field work is to use transparent plastic vials with screw tops (Fig. 3). Alternative preservation fluids which may depend on the taxonomic group or the purpose of the field work can be found in Appendix 1. In general, formalin is not recommended for collecting and preserving arthropods. Specimens become very rigid which complicates the handling. In some cases

this effect may be desirable, as for soft-shelled specimens or larval instars or for specimens that are intended to be included in anatomical dissections.

Fig. 2. Handling Glass vials are not a good choice for storing specimens during field work as they are heavier and less safe than plastic vials. (Photo by authors).

Fig. 3. Plastic vials are safer than glass vials during field work and also less heavy. A screw top with a ring gasket tightly closes the vials and prevents evaporation of the ethanol. The transparency of the vials allows the collector to check the content and labels without the need to re-open the vials. (Photo by authors).

3.3.2. Dry-mounting of insects after fluid fixation

The used fluid can be of great importance if the collector's intention is to dry-mount specimens after fixation in a preservation fluid. Acetic acid (2%) is recommended for well sclerotised taxa like beetles (Coleoptera), bugs (Heteroptera), and ants (Formicidae). Specimens in ethanol mostly become rigid and handling and mounting is complicated. Better results are only accessible via more elaborate methods like chemical treatment, heat impact or critical point drying. Inapplicable for dry-mounting after fluid fixation are Lepidoptera, Diptera, as well as pilose and coated Hymenoptera. Micro-Hymenoptera as well as any other small and delicate insect specimens should only be mounted after critical point drying. In these cases it is necessary to transfer them along an ethanol series in the laboratory, in which the ethanol concentration is gradually increased from 70-80% via 90% and 95% to absolute ethanol. Also for some insect larvae and small arachnids it can be more advisable to mount them after critical point drying instead of storing them permanently in ethanol.

3.3.3. Standard methods for dry preservation and mounting of insects

The standard method for dry preservation of well sclerotised insects is the use of specific insect pins. All characters of the specimen should be readily visible by mounting it in a characteristic manner like spreading wings and limbs. There are some different setting and mounting recommendations according to the taxonomic group to be mounted.

Dry-mounting and pinning is recommended or even necessary for the following groups:

- Lepidoptera
- Coleoptera
- Hymenoptera
- Diptera (partim: most Brachycera, single Nematocera groups)
- Heteroptera
- Saltatoria and other "Orthoptera"
- Odonata (imagines and exuviae)
- Neuropterida (partim)

All other taxonomic insect groups as well as insect larvae, Arachnida, Myriapoda, and Crustacea are best killed and preserved in 70 -80% ethanol.

There are 3 established alternatives of pinning (with some modifications in special cases) which are determined by the specimens' dimensions:

- Direct pinning of the specimen with commercially available insect pins of different size for well dimensioned objects.

- Direct pinning of the specimen with commercially available minuten pins of different sizes for small specimens (standard method for Micro-Lepidoptera). Minuten pins are to be pinned into small double mount strips, which are again pinned with common insect pins (see Schauff, undated, for more information).

- Gluing the specimens onto small paper cards of different size and shape. This method is also referred to as card-mounting.

Direct pinning of specimens should be done with an insect pin that fits to the specimen's size. The standard size for insect pins is 1 or 2 which fits for most Lepidoptera, large Hymenoptera and many Coleoptera. Larger specimens should be pinned with size 3, 4 or 5, while for smaller specimens, pins with size 0, 00, or even 000 are available. However, it should be noted that pins with size 0 or smaller are difficult to handle. Pinning through the labels or through the paper layer of insect boxes should be done with great care as the thin pins are easily twisted. Therefore, it may be more appropriate to use minute pins or glue for very small specimens. Direct pinning of insects should be done in a way that the pin is in a right angle to the body (Fig. 4). The insect specimens should rest about 1/3 of the pin length away from the top. This gives enough space to handle the specimens, *i.e*. to grip the top of the needle by the thumb and the index finger without damaging the specimens with the fingertips or fingernails. The specimens should not rest further away from the top of the needle as the bottom space is needed for collection and determination labels. The pin is usually inserted through the mesothorax but the exact insertion point depends on the insect group (Figs 5A-D). Bugs (Heteroptera) are pinned submedially through the scutellum (Fig. 5A). In Hymenoptera and Diptera the insertion point is slightly removed laterally from the median axis (Fig. 5B). This allows median sculpture or bristle patterns to remain intact and visible medially and also on one side. Beetles (Coleoptera) are pinned through the right elytron (Fig. 5C). In butterflies and moths (Lepidoptera) the insertion point is in the middle of the mesothorax (Fig. 5D).

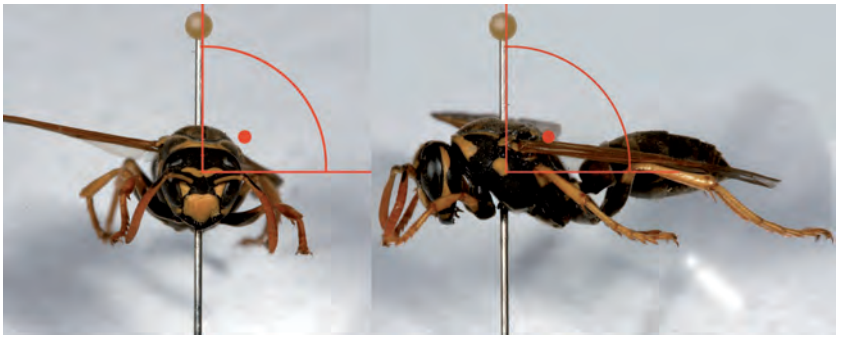


Fig. 4. While pinning an insect specimen care must be taken that the needle is in a right angle to the body of the insect. This needs to be checked in frontal and lateral view. (Photo by authors).

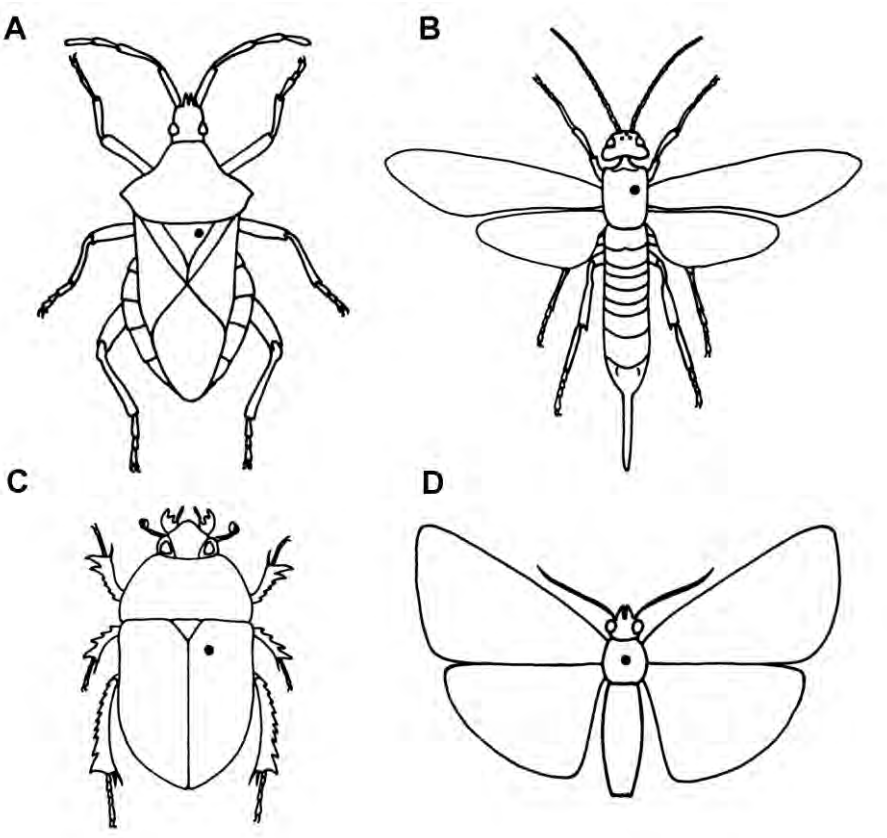


Fig. 5. Insertion of the insect pin, as exemplified in the orders of A. Heteroptera; B. Hymenoptera; C. Coleoptera; and D. Lepidoptera (after Abraham, 1991). See text for more details.

Very small insects (body length below 3 mm) should never be directly pinned on minutens as specimens will always be damaged or lost over time. Card-mounting is the method of choice for small beetles, bugs and Micro-Hymenoptera. Beetles and bugs are usually glued on rectangular cards (Fig. 6). Small Hymenoptera can either be glued on rectangular cards or on the tip of card points, which are small triangles of stiff paper (Fig. 7) (Noyes, 1982, 2009). The latter method has the advantage that the specimen can also be observed in ventral view. The paper cards with the mounted insects are pinned with common insect pins of larger size (sizes 3 to 5). Pins of that size can easily be inserted through the paper cards. The glue should be water-soluble or ethanol soluble so that specimens can easily be removed from the card in case they need to be re-mounted without being damaged. Noyes (2009) recommends glues, which were made from animal products. The best option is to use shellac, a resin produced by lac bugs (Coccoidea). It is important to use shellac (or any other glue) in the right solution, *i.e*. the glue should not be too thin (the specimen will sink in the glue) or too thick (the specimen will not attach tightly enough). Shellac can easily be brought to the right viscosity by adding drops of ethanol or by letting part of the ethanol evaporate from the glass tube in which

the resin is deposited. Shellac is commonly used in North America but less widespread among European entomologists. Shellac resin can be obtained from entomological suppliers in the United States. However, even if we would recommend shellac over other glues, there are also a few drawbacks of shellac which are best summarized in Noyes (2009). Seccotine (fish glue) is a water-soluble glue and a good alternative to shellac. Noyes (pers. comm.) recommends the use of shellac for card points and seccotine for card rectangles. For long and slender insect groups, *e.g.* ichneumonid wasps, it can be an alternative to glue them on to the side of an insect pin with shellac.

Fig. 6. Card mounting is the method of choice for small sized insects. Small beetles are usually mounted and glued on rectangular paper cards, which are available in various sizes from entomological suppliers. (Photo by authors).

Fig. 7. Card points are small triangles of stiff paper that allow specimens to be observed from all sites if specimens are glued laterally to the tip of the triangle. This is a suitable method for mounting Micro-Hymenoptera. (Photo by authors).

Accepted killing methods for arthropods to be dry-mounted are:

- Jar, containing absorbent paper saturated with ethyl acetate (Fig. 8B). This method is suitable for most insects apart from Lepidoptera. Avoid too wet content of the jar because of possible agglutination of small, pilose, or coated specimens.
- Potassium cyanide inside a killing jar (Fig. 8D). This is best method for Lepidoptera, except for some resistant groups like Zygaenidae moths. It is also feasible for most other taxonomic groups. Safety regulations are essential to avoid intoxication! It is the responsibility of the collector to make sure that the killing jars are always kept under supervision and do not get into the hands of others!
- Ammonium chloride, injected via syringe (Fig. 8C). This can be used for larger butterflies and moths effecting rapid killing and for softening rigour mortis.
- Freezing.

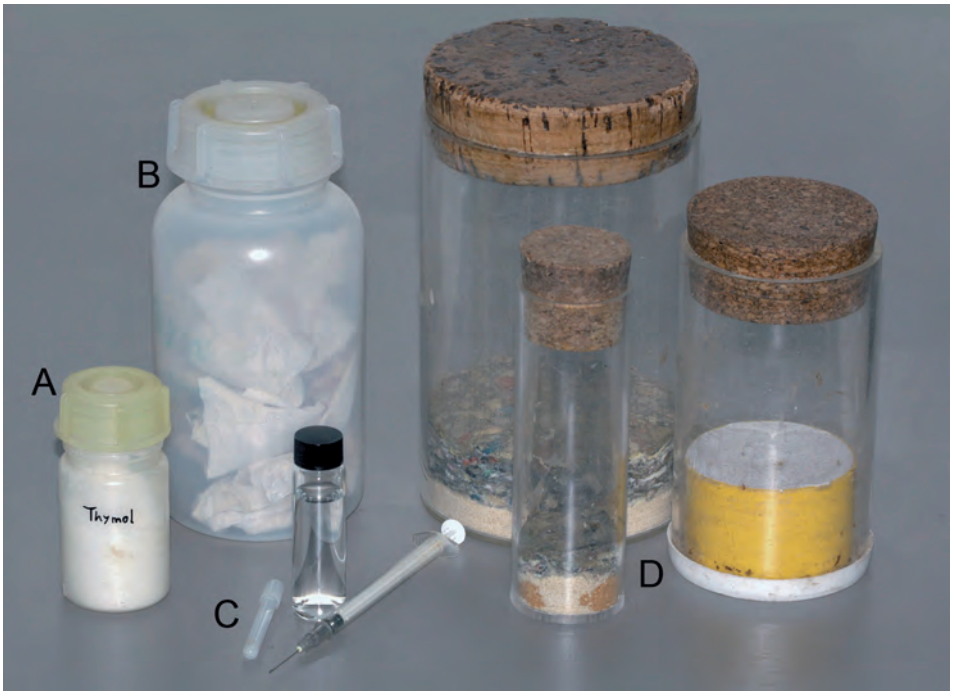


Fig. 8. Devices against moulding and for killing specimens. A. Thymol prevents specimens from fungal damage and can be applied crystalline or in a solution; B. Ethyl acetate is used for most insects apart from Lepidoptera; C. Ammonium chloride is used for larger Lepidoptera and injected by a syringe; D. Killing jars containing potassium cyanide can be purchased from entomological suppliers in various sizes. Specimens are usually killed in smaller jars and transferred into a large jar after they are narcotized. Layers of tissue between the specimens prevent them from mechanical damage during fieldwork. (Photo by authors).

3.3.4. Preservation in the field and transport

If time availability and the amount of samples permit, it is good practice to mount or prepare for dry preservation.

Even if this is not possible, the right preparations and transportation conditions are essential for best quality of set specimens. When pinning and mounting sheetings and/or setting boards, transport and store boxes are available they should be used already in the field. Protected and dry storage is important; especially intruding ants could be fatal. In many cases setting in the field is not possible. In these cases, the following recommendations should be noted to facilitate later setting and mounting:

- Use cardboard tubes of different diameter for transport (Fig. 9). Both sides of a tube are to be closed with a cotton plug. The freshly killed sample is placed directly inside the tube. It can be transferred into a soften chamber afterwards for preparation of setting and mounting. Check adequate labelling! This method is feasible for strongly sclerotized specimens (*e.g.*, beetles) which need to be stored during fieldwork before they can be dry-mounted in the laboratory. However, scaled, pilose, and coated specimens could be rubbed off during transport. Cardboard tubes are preferred over glass or plastic ones as they are lightweight, fracture-proof and absorb moisture. The tubes are to be stored inside of feasible sealed transport boxes containing crumbs of thymol (Fig. 8A) against moulding.

- Use butterfly envelopes of different size, made of vellum (Figs 10, 11). This is the best method to transport or even store dry unset Macro-Lepidoptera, but also feasible for other winged insect orders like Odonata and Neuroptera. It is important to “close” the specimens inside of the envelope with the wings folded upwards. This protects the more important upper sides of the wings (as identification characters) against rubbing and facilitates later setting and spreading. If this is not possible in case of *rigor mortis*, the specimens have to be injected by syringe with ammonium chloride to soften *rigor*. Placing more than one specimen into one envelope should be avoided as they may damage each other during transport. Every single envelope has to be labelled individually with the full locality data! The envelopes are to be stored inside of sealed transport boxes containing crumbs of thymol against moulding (Fig. 11). For softening the specimens the whole envelope has to be transferred into the soften chamber without removing its content.

- Use small plastic boxes laid out with layers of cellulose wadding. Freshly killed insect specimens can be placed between the layers and will be protected during transport. Thymol should be added against fungal damage.

- Use transport boxes with plastazote foam pinning bottoms (Fig. 12). Insects can be pinned without setting and plunged into the box in a space-saving manner. For later setting, mounting or spreading, they can be softened easily inside a soften chamber. This is feasible for all well sized specimens, which are to be pinned and set. Adding labels to every single specimen is essential! Fragile Micro-Lepidoptera that cannot be transported on setting boards (this is the preferred option) should be pinned directly onto plastazote in small transport boxes (Upton, 1991). Spreading the wings with

minuten pins can easily be done in the field and the roughness of the plastazote will hold the wings in place (Fig. 12). This method does not replace proper spreading on a setting board but will greatly facilitate this as the wings are already partially spread.

Fig. 9. Cardboard tubes are ideal for hard bodied insects, such as beetles. Specimens are ideally protected during transport and less prone to moulding as the tubes absorb moisture. (Photo by authors).

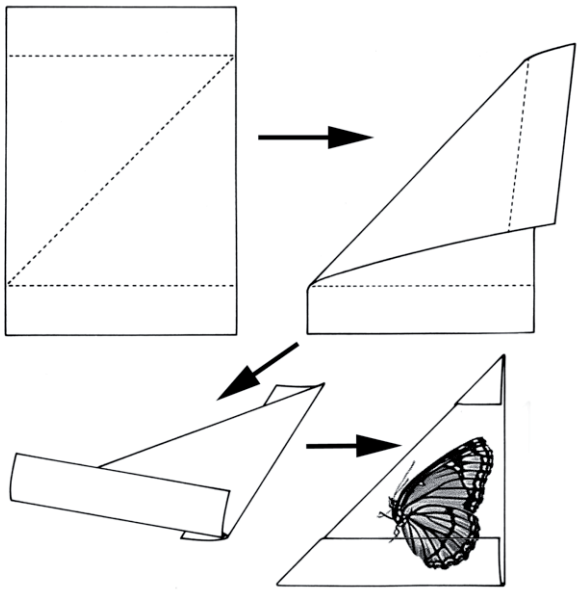


Fig. 10. Envelopes for storing insect groups, such as Lepidoptera can be easily folded from rectangular paper (after Abraham, 1991).

Fig. 11. Vellum envelopes are a simple option for storing Lepidoptera specimens as they do not need to be hand-folded but can be readily purchased in various sizes from philately purchasers. The envelopes are best stored in tightly lidded boxes which can be laid out with wet cotton. The moist atmosphere keeps the specimens relaxed before mounting. Thymol must be added to prevent moulding. (Photo by authors).

Fig. 12. Transport boxes with plastazote foam pinning bottoms are ideally suited to transport pinned insects in the field. Fragile Micro-Lepidoptera that cannot be transported on setting boards should be pinned directly with minutens onto the plastazote. The wings should be spread and the roughness of the plastazote will hold them in place. This greatly facilitates later spreading on a setting board. (Photo by authors).

4. Acknowledgements

We thank Daniel Bartsch and Axel Steiner (Staatliches Museum für Naturkunde Stuttgart) for helpful discussions and advise on preservation of Lepidoptera.

5. References

ABRAHAM, R. 1991. *Fang und Präparation wirbelloser Tiere*. G. Fischer, Stuttgart, New York: 132 pp.

KLEYNHANS, K.P.N. 1999. *Collecting and preserving nematodes. A manual for nematology*. ARC Plant Protection Research Institute, Pretoria, South Africa: 54 pp. Available at: http://spc.int/pps/SAFRINET/nem-scr.pdf; accessed 28 September 2009.

KNUDSEN, J.W. 1972. *Collecting and preserving plants and animals*. Harper and Row, New York, Evanston, San Fransisco, London: 320 pp.

NOYES, J.S. 1982. Collecting and preserving chalcid wasps (Hymenoptera: Chalcidoidea). *Journal of Natural History* 16: 315-334.

NOYES, J.S. 2009. Universal Chalcidoidea Database. Collecting and preserving chalcidoids.http://www.nhm.ac.uk/researchcuration/research/projects/chalcidoids/collecting1.html; accessed 28 September 2009.

PIECHOCKI, R. & HÄNDEL, J. 2007. *Makroskopische Präparationstechnik Wirbellose. Leitfaden für das Sammeln, Präparieren und Konservieren*. 5th Ed. E. Schweizerbart, Stuttgart: 346 pp.

SCHAUFF, M.E. undated. Collecting and preserving of insects and mites. Techniques and Tools. http://www.ars.usda.gov/SP2UserFiles/ad_hoc/12754100CollectingandPreservingInsectsandMites/collpres.pdf; accessed 28 September 2009.

STURM, C.F., MAYHEW, R. & BALES, B.R. 2006. Field and laboratory methods in malacology. *In*: STURM, C.F., PEARCE, T.A. & VALDES, A. (Eds) *The molluscs: A guide to their study, collection, and preservation*. Universal publishers, Boca Raton, FL, USA: 445 pp.

UPTON, M.S. 1991. *Methods for collecting, preserving, and studying insects and allied forms*. 4th Ed. The Australian Entomological Society, Brisbane: 86 pp.

6. Appendix I: Reagents and solutions suitable for field work

Acetic acid-glycerol-alcohol solution (AGA) (fixation of small arthropods and thrips)
1 part of glacial acetic acid
6 parts of ethanol (95%)
4 parts of H_20
1 part of glycerol

Alcoholic thymol-camphor solution (prevention of mould)
100 ml ethanol (96%)
5 g camphor (crystalline)
10 g thymol (crystalline)

Bouin's solution (fixation of insect larvae for histological work)
70 parts of picric acid solution
25 parts of formalin
5 parts of glacial acetic acid

Formal-acetic-alcohol (FAA) (fixation of flatworms and other animals)
10 parts of formaldehyde solution (saturated) (= 100% formalin solution)
50 parts of ethanol (95%)
2 parts of acetic acid
40 parts of H_20

Formalin
Refers to a saturated solution of formaldehyde. Formaldehyde comes in a saturated solution of 39-40% which equals a 100% formalin solution. That means that *e.g.* a 10% percent formalin solution can be obtained by adding 1 part of formaldehyde (saturated) to 9 parts of water.

Formol-alcohol (fixation of some annelids)
1 part formol
2 parts ethanol (80%)

Kahle's solution (= Pampel's fluid) (general fixation of insect larvae)
30 ml ethanol (95%)
10 ml formalin (35-40%)
2 ml glacial acetic acid
60 ml H_20

Lactic alcohol (for aphids and scale insects)
2 parts of ethanol (95%)
1 part of lactic acid (75%)

Chapter 19

Field Methods and Techniques for Monitoring Mammals

by

Anke Hoffmann
Museum für Naturkunde Berlin
Invalidenstr. 43, 10115 Berlin, Germany
Email: anke.hoffmann@mfn-berlin.de

Jan Decher
University of Vermont
Burlington, Vermont 05405, USA
Email: jan.decher@uvm.edu

Francesco Rovero
Museo Tridentino di Scienze Naturali
Via Calepina 14, 38122, Trento, Italy
Email: francesco_rovero@yahoo.it

Juliane Schaer
Museum für Naturkunde Berlin
Invalidenstr. 43, 10115 Berlin, Germany
Email: jule.schaer@gmx.de

Christian Voigt
Leibniz-Institute for Zoo and Wildlife Research
Alfred-Kowalke-Str. 17, D-10315 Berlin, Germany
Email: voigt@izw-berlin.de

Gudrun Wibbelt
Leibniz-Institute for Zoo and Wildlife Research
Alfred-Kowalke-Str. 17, D-10315 Berlin, Germany
Email: wibbelt@izw-berlin.de

Abstract

This chapter is a brief introduction to inventory methods for mammals in terrestrial habitats, with a focus on trapping methods for terrestrial small mammals, bats and medium-sized (meso-) mammals. For large mammals we refer the reader to more detailed sources. We suggest guidelines for designing a study, introduce selected trapping and handling procedures, and make recommendations for field equipment and data recording. Practical notes and hints based on authors' field experience are integrated in all sections of the chapter. Additionally, the authors review safety precautions and cover practical aspects for what to do "before launching" an expedition.

Key words: animal handling, bats, preservation, small mammals, trapping

1. Introduction

Aim of this chapter is to describe inventory methods for mammals in terrestrial habitats, with an emphasis on small- and medium-sized (meso-) mammal and bat surveys through a variety of trapping methods. We provide guidelines for designing a study, specify trapping and handling procedures, and make recommendations for field equipment and primary data records. Practical notes and hints based on our own field experience are integrated in all sections of the chapter.

For practical aspects the outline of the chapter is based on three non-taxonomic groupings. Due to the difference in mammal body size and their mode of life, such as volant and non-volant, specific methods, techniques and approaches are required and are therefore treated separately. We give a brief introduction on mammalian diversity and define operational terms for “Small-, Medium-sized and Large Mammals”.

1.1. Mammalian diversity

The Class Mammalia can be coarsely divided as follows (Simpson, 1931):

- Subclass Prototheria (Monotremes, one Order)
- Subclass Theria
    - Infraclass Metatheria (Marsupial Mammals, seven Orders)
    - Infraclass Eutheria (Higher Mammals - Placentalia; 21 Orders)

According to the most recent (3rd) edition of the standard taxonomic reference work, *Mammal Species of the World* (Wilson & Reeder, 2005; hereafter: MSW3), the class Mammalia comprises 5416 species (Tab. 1). Of these 2277 (42 %) are rodents (Rodentia), 1116 (20.6 %) are bats (Chiroptera) and 428 (7.9 %) are shrews and allies (Soricomorpha). However, these numbers are a taxonomic “snapshot” in time. Taxonomy is extremely dynamic, especially since the advent of molecular genetics has accelerated the revision of taxonomic groups and species delineations, but also with increased efforts to survey the last undisturbed places in a race against accelerating extinctions (Wilson, 1992).

MSW3 includes 787 more species than the 2nd (1993) edition, 260 species of which are newly described species. Ten of these were large mammals (8 artiodactyls, 1 carnivoran, and 1 whale). The vast majority were small mammals: 49 bats, 18 soricomorphs (17 shrews, 1 solenodont), and 128 rodents. This rapid increase in known mammal species highlights the importance of continued, standardized survey work throughout the world, particularly in habitats that are little known and/or in danger of being destroyed due to logging, mining, or other forms of “development“. Since much of All Taxa Biodiversity Inventory and Monitoring (ATBI+M) focuses on biodiversity surveys, conservation assessments and baseline data collections, we recommend using MSW3 (or its subsequent editions) as a taxonomic standard and referring to more recent taxonomic changes in the primary literature only in specific cases. For more information on mammalian diversity and natural history consult Cole & Wilson (1996).

Order	No. of Species	% of Total	Order	No. of Species	% of Total
Rodentia	2277	42.04	Scandentia	20	0.37
Chiroptera	1116	20.61	Perissodactyla	17	0.31
Soricomorpha	428	7.90	Macroscelidea	15	0.28
Primates	376	6.94	Pilosa	10	0.18
Carnivora	286	5.28	Pholidota	8	0.15
Artiodactyla	240	4.43	Paucituberculata	6	0.11
Diprotodontia	143	2.64	Monotremata	5	0.09
Lagomorpha	92	1.70	Sirenia*	5	0.09
Didelphimorphia	87	1.61	Hyracoidea	4	0.07
Cetacea*	84	1.55	Proboscidea	3	0.06
Dasyuromorphia	71	1.31	Notoryctemorphia	2	0.04
Afrosoricida	51	0.94	Dermoptera	2	0.04
Erinaceomorpha	24	0.44	Microbiotheria	1	0.02
Paramelemorphia	21	0.39	Tubulidentata	1	0.02
Cingulata	21	0.39			
			Total Number of Mammal Species	**5416**	

Table 1. Mammalian Orders based on Mammal Species of the World 3rd Edition listed in descending order of species diversity with percentage of total mammal species. *Two orders are comprised entirely of species that are highly adapted for life in aquatic (primarily marine) environments.

1.2. Definition of "Small, medium-sized and large mammals"

We will frequently refer to small and medium-sized mammals not as taxonomic groupings, but as practical subdivisions that require different methods and approaches.

"Small mammals" are usually divided into small terrestrial and volant mammals (bats). Terrestrial small mammals commonly refer to everything smaller than the largest rodents (capybara, nutria, grasscutter) or lagomorphs (hares, rabbits and pikas). Although some authors *(e.g.* Bourlière, 1975; Stoddart, 1979; Gaines & McClenaghan Jr., 1980) include in "small mammals" all mammal species, whose weight or size is less than a hare (3-5 kg), we include in "small mammals" only species weighing less than 500 g, the upper size limit that can easily be caught in commercially produced live traps (see Section 2.2) used in a standard small mammal survey. There is a considerable range of species within

this limit, including shrews, moles, most rats, mice, lemmings, gerbils, jerboas, dormice and many squirrels (Delany, 1974).

"Medium-sized mammals" is often used for small carnivores, small primates, large rodents, hyraxes, and pangolins that are not adequately covered by small mammal trapping arrays and require larger (wire mesh) traps. Some of these mammals can also be detected through non-trapping "observational" methods, such as track censuses or automatic camera traps. This group includes some of the most secretive, hard to survey, and hence still poorly known species.

"Large mammals" include most diurnal primates, most carnivores larger than a fox or house cat, all perissodactyls (horses, rhinos, tapirs) and artiodactyls (including the relatively small duikers). There will be some overlap between these broad categories. For example, in North America the smaller weasels (*Mustela* sp.) are caught in traplines set for rodents and shrews (about 1 weasel per 200 rodent captures, J. Decher, unpubl. data), but these traps exclude the larger mustelids like mink *Neovison* (*Mustela) vison* or Marten and Fisher (*Martes* sp.). In Africa the largest rodents (*Atherurus, Cricetomys, Thryonomys*, etc.) are best caught in large wire traps (Tomahawk, Havahart) and often show up on automatic camera trap pictures.

In section 2.1 we provide a brief summary of field techniques for medium-sized mammals in species inventories. Medium-sized mammals are generally much less known than larger mammals. We do not address the vast methodology on large mammals, because it is adequately presented elsewhere (*e.g.* Caughley, 1977; Davis, 1982; Wilson *et al.*, 1996; Martin *et al.*, 2000). A specific chapter in this volume is dedicated to camera-trapping (see Chapter 6), which in recent years has been developed into an efficient method for surveying both medium-sized and large mammals.

2. Field techniques

2.1. Medium-sized mammals

2.1.1. Trapping methods

Trapping of medium-sized mammals is generally more challenging and costly than trapping of small mammals, and therefore it is more often used for focal studies (*e.g.* radio-tracking or collection of DNA samples) than for species inventories. Moreover, some species or groups of species will require *ad-hoc* trapping methods: for example, small nocturnal primates can be trapped using Chardonneret traps (15 x 15 x 23 cm) baited with bananas and placed in trees (Doggart *et al.,* 2006); the larger elephant-shrews of the genus *Rhynchocyon* have been successfully trapped using fishing nets strung along the forest floor (Rathbun, 1979). For general trapping methodology and procedures we refer to the detailed account on small mammals presented in Section 2.2. Suitable traps for medium-sized mammals are commercially available, *e.g.* Tomahawks and Havaharts (details in Section 2.2). They consist of foldable cages made of galvanized wire mesh and can be single or double-door; the size varies depending on target species, with 18 x 18 x 61 or 23 x 23 x 66 cm being a

standard size for trapping small carnivores and large rodents. Because of limited survey budgets, it is rarely possible to purchase large numbers of these traps and therefore trapping will be more successful when animal's trails, nests or burrows can be found, which in tropical countries is usually facilitated by local hunters. Medium-sized mammal traps need to be checked very frequently as captured animals may become stressed quickly and hurt themselves trying to bite or dig through the mesh.

2.1.2. Observational methods

"Observational methods", including camera-trapping, can be used to survey some groups of medium-sized mammals. There are three types of observational methods: (1) direct observations, (2) identification of dung, tracks and other signs, and (3) camera-trapping, *i.e.* the use of remotely set, automatic cameras. Because of the more challenging technological implications and recent advances and applications in camera-trapping, this method is described in full detail in Chapter 6. There are firm indications that camera-trapping is a very cost-efficient method of surveying both medium-sized and large mammals, especially in forest habitats, where visibility and track/dung detection may be difficult (see Chapter 6 and Bowkett *et al.*, 2006). Nevertheless, both direct sightings and signs should be considered either as an alternative method or to complement camera-trapping surveys.

In general, direct observation is not an efficient method to detect medium-sized mammals. There are a few exceptions, for example the nocturnal primates, whose eye shine is easily sighted at night using head torches (Doggart *et al.*, 2006). Some of the locally common small African carnivores, such as palm civets and genets, can also be sighted with torches during night walks. Tracks, scats and other signs can, instead, be more easily recorded in the course of any survey. Photographs, possibly including a scale reference, can assist with later identification confirmations, and localities should be recorded with a handheld GPS unit. Sometimes indigenous knowledge (especially from hunters) can be useful for a preliminary list of species and/or help with identification of signs. Chances to detect tracks can be increased with tracking stations, by clearing portions of an animal trail and covering the ground with fine sand or with special track plates with surface blackened using smoke or printer toner (Zielinski, 1995; Wemmer *et al.*, 1996; Foresman & Pearson, 1998). Synthetic attractant or natural lures can be placed at tracking stations, especially to attract carnivores.

An introduction to mammalian signs including tracks, nest and burrows, scats and food caches is provided by Wemmer *et al.* (1996). A more detailed treatment on tracking in the North American temperate environment is found in Rezendes (1999). Field guides to the identification of signs and tracks for different parts of the world can be found in corresponding specialised field guides for mammals (Stuart & Stuart, 1994; Rezendes, 1999; Bang & Dahlstrom, 2001; MacDonald & Barrett, 2002; Ohnesorge & Scheiba, 2007).

2.1.3. Indirect methods

Indirect surveys of small carnivores may involve hunting or fur harvest records such as the classic study of Canada lynx (*Lynx canadensis*) cycles and more recent work on mink (*Neovison vison*) in Canada (Elton & Nicholson, 1942; Shier & Boyce, 2009), surveys of meat markets in Africa (Anadu *et al.*, 1988; Angelici *et al.*, 1999; Crookes *et al.*, 2005), or setting up scent marking stations with hair traps to monitor small carnivores (Schmidt & Kowalcyk, 2006).

2.2. Small mammals

Even though terrestrial small mammals are often quite abundant, they are rarely observed and (except in snow or sand) their tracks are rarely seen and hard to identify to species. However, they can be easily sampled with sufficient numbers of traps or pitfalls, and the most abundant species in a small mammal assemblage allow for population estimates using capture-mark-recapture protocols (Smith *et al.*, 1975; Caughley, 1977; Krebs, 1989). Most small mammals are easily handled requiring relatively little specialized equipment.

2.2.1. Traps and bait

For most terrestrial small mammals the Sherman live trap (http://www.shermantraps.com) has become the standard foldable, very portable and efficient trap of choice (Fig. 1). H. B. Sherman makes several sizes and has recently started to offer most models with perforated walls, which should help prevent overheating in hot grassland or semi-desert environments.

The standard model (LFA-TDG, 7.5 x 9 x 23 cm) is the most widely used trap, especially in the United States. In tropical environments we have found the extra long model (XLK, 7.7 x 9.5 x 30.5 cm) to be preferable given the larger average size of tropical mammals and the long tails of many genera (*e.g. Malacomys, Dephomys*). If only the standard model is available, but capture of long-tailed species is expected, traps could be modified to avoiding injury of the animals by attaching a small spacer piece at the top rim of the trap which creates a narrow gap (*ca.* 3 mm) for the animals to safely pull their tails into the trap. The largest model (XLF15, 10 x 11.5 x 38 cm) has been tested successfully by one of us (J. Decher) with small mammals. It should theoretically be useful in a study focusing on small mustelids or herpestids, but many medium-sized mammals can be extremely shy to enter a trap with solid walls and a wire or cage trap like the Tomahawk models (http://www.livetrap.com) is preferable.

In colder climates the small mammal trap of choice may be one that has a nest box attached, such as the British Longworth trap (Penlon Ltd., Oxford, U.K., http://www.alanaecology.com). However, the Longworth design is not collapsible, and the traps are considerably more expensive than the standard Sherman trap. The usability of Sherman traps in colder climates can be extended by placing the traps into “waxed cardboard” containers (Fig. 2) saved

Fig. 1. Popular trap types. Back: Collapsible Tomahawk trap for squirrels, small carnivores, and large rats. Centre: Standard-sized collapsible Sherman trap. Front left: Victor Rat trap. Front right: Museum Special snap trap. (Photo by Jan Decher).

Fig. 2. Standard Sherman Live Trap (LFA-TDG, 7.5 x 9 x 23 cm) protected in a 2 litre milk carton. (Photo by Anke Hoffmann).

from milk or juice products. Some bedding like cotton or shredded paper can be stuffed into the very back of Sherman traps as well as additional food, as long as it does not block the treadle mechanism. In general, live trap survival can be improved if covers are used to protect traps from the elements (sun, snow, rain).

In one comparison of small non-folding (5.4 x 6.5 x 17 cm), and large (7.7 x 9.1 x 23 cm) folding Shermans with two-piece Longworth traps (13.8 x 6.4 x 8.4 cm), small Shermans captured the most animals and appeared to be the most effective traps for smaller-sized mammals. Longworth and Sherman traps exhibited species-specific differences in capture rates suggesting that they should be used in combination to reduce overall bias (Hoffmann, 1995; Anthony *et al.*, 2005). Similarly Nicolas & Colyn (2006) compared the efficiency of Sherman traps, metal snap and pitfall traps and concluded that an assortment of traps should always be employed in studies of small mammal communities in African rainforest in order to obtain a wider range of taxa, and thus a better representation of the community.

Larger wire traps are offered in numerous sizes, and in single or double door and rigid or collapsible versions by the Tomahawk (http://www.livetrap.com, see Fig. 1) and Havahart (http://www.havahart.com) trap companies. Some rapid biodiversity assessments when maximum trap success is important may justify the use of snap traps of various types. Because a standard mouse trap from the hardware store is often too weak for wild rodents and shrews and the larger rat trap is too large for smaller species, a medium sized trap was developed known as the “Museum Special” trap (Fig. 1). It also has a better probability for leaving small mammal skulls (the most important museum-diagnostic structure in mammals) intact (Smith *et al.,* 1971, but also see Perry *et al.,* 1996). When trapping in protected areas check with authorities if the use of removal traps is permitted.

Recent studies have emphasized the need to avoid bias towards certain species by trapping only on the forest floor in tropical environments. For this reason a number of workers have taken to placing traps on platforms that can be lowered with a pulley system high in the canopy to sample for scansorial or arboreal species. However, initial placement of the trap platforms (or pulley attachment) requires special climbing gear and considerable athletic skills (Malcolm, 1991, 1995; also see Jay Malcolm checking his arboreal traps in the video *Rain Forest*, National Geographic Society, 1998).

The use of bait versus no bait and the advantages of pre-baiting (baiting for several days prior to placing or setting the traps), when survey time allows for it, have been discussed elsewhere (Smith *et al.*, 1975; Jones *et al.*, 1996). Numerous favourite recipes exist on the subject of bait preparation. Standard bait among many mammalogists is oatmeal flavoured with peanut butter. We have also known a mammalogist who routinely chewed (!) the oatmeal to prepare it for use on snap traps. One of us (A. Hoffmann) prepares a “sticky cake” from oatmeal, peanut butter (or locally sold “groundnut paste” in Africa) and bananas, if available, which can be formed into adhesive balls that can easily be attached to the back of Sherman traps or on the treadle of a snap trap. Another effective recipe, if no peanut butter is available, is a sticky dough made from maize flour, ripe bananas and (roasted) peanuts (Hoffmann, 1999). We

have also successfully used shavings of the outer fibrous and oily (pericarp) layer of oil palm nuts (*Elaeis guineensis*) in Africa. Their scent seems to equally attract insectivorous shrews and rodents.

Pitfall traps may be the most effective trap for mammals under 10 g. Pitfalls can be made from 5-10 litre buckets, large yogurt containers or specially made cones (Pankakoski, 1979). Cones are very useful in marshy habitat where they can just be pushed into the ground. In rocky or laterite soil, pitfalls can mean a large investment of labour, but their placement is often rewarded by the capture of small shrews not sampled with any other method (Handley & Kalko, 1993; Kalko & Handley, 1993; Nicolas & Colyn, 2006). Pitfalls work most efficiently if they are connected by a plastic or mesh drift fence running across each pitfall. For an example of a very thorough application of pitfalls and drift fences to shrew diversity and abundance in different habitats in Guinea, see the recent work by Nicolas *et al*. (2009).

Pitfalls work well unbaited but we have also baited them in certain situations. They should be checked often to avoid multiple animals captured from attacking each other or being taken by predators. Buckets should be punctured to reduce the chance of drowning during heavy rains. Buckets can also be covered with small boards spaced with a gap above the buckets using three or four rocks to reduce flooding and predator impact. Some plant material and little stones in the bucket can also provide hiding places and protection against sun and rain for the animals. For pitfall traplines shorter spacing distances ($\leq$ 5 m) have been recommended, because of the smaller size of the target species (Handley & Kalko, 1993). The array of a pitfall trapline depends much on habitat, substrate and man power. The length per line can vary between 10-50 m, whereas the set-up of the drift fence must still be practicable. Some workers have recommended more elaborate drift fence and pitfall arrays such as a Y-shaped design with a pitfall at each end of the fence arms and one in the centre (Kirkland & Sheppard, 1994). Pitfall set-up and results can sometimes be shared with entomologists and herpetologists who might be working on the same inventory (see Chapters 9, 14 & 20).

2.2.2. Trapping procedure

The way traps are arrayed in the habitat depends on the question being asked and the estimation methods used. For inventories, accurate estimates of abundance (total number of animals) or density (numbers per unit area) are not necessary: the primary concerns are assessing the true mammal diversity of an area by sampling a sufficiently large area with a diverse array of methods. In any case, a standardised design should be used and carefully documented to allow for future repetition and facilitate a meaningful long-term monitoring effort.

Trapline designs

For inventories of small terrestrial mammals the easiest approach is to place traps at equal intervals along a line, which ideally should cover all habitat types, ideally with one or two replicate lines. Spacing distances are a function of habitat complexity. Traps in more complex habitats should be more closely

placed. Size of the target species is also a consideration, because smaller mammals tend to travel shorter distances than larger mammals (Jones *et al.*, 1996). We recommend that a trapline ideally be about 150 m long, with traps placed every 10 to 15 m (Mühlenberg, 1993; Jones *et al.*, 1996), but this design has to be adapted to the respective habitat conditions and target species. Whatever the spacing, to increase the trap success, traps should be placed at habitat features (*e.g.* log, rocks, tree, runways, burrows, bush clusters) as long as they lie within 2 m of the point. Where possible, a subset of traps should also be placed on branches of trees in order to catch scansorial species. If freshwater habitat (stream, pond, lake) is present, we recommend placing several traps near these bodies of water. Traplines near water and in trees should be tethered to reduce loss due to sudden water level changes or traps falling out of trees. For replicate traplines, we recommend a minimum distance of at least 100 m between the traplines to avoid an impact on the trap success.

Trapping effort is commonly expressed in "trap-nights", that is the number of traps multiplied by the number of daily trap periods (*e.g.* sunset to sunrise). A minimum of 400-500 "trap-nights" has been recommended for a preliminary inventory of a habitat (Jones *et al.*, 1996; Fraser *et al.*, 2003). Thus, at least 100-150 traps are needed for an efficient inventory survey so that the trapping period can be limited to three or four consecutive nights in each habitat and season. More traps reduce the number of daily trap periods, but are difficult to check efficiently in one trap inspection especially if many measurements and habitat data are recorded at each trap station. The required trapping effort can be determined with a species accumulation curve (Colwell *et al.*, 2004; Decher *et al.*, in press).

We recommend placing two traps at every station to reduce the saturation of traps by "trap-happy" individuals or very abundant species. This practice increases the chance trapping animals that are less active, less attracted to traps (Drickamer, 1987), or "trap-prone" (Andrzejewski *et al.*, 1971). Each trap station should have at least one Sherman trap, which can be combined with any other trap type available. If 80% of the traps are occupied it is recommended to increase their number (Corbet & Harris, 1990).

Whether traps follow a rigid grid arrangement or a linear trapline, individual traps can often be set opening towards, or in line with a rodent runway, along a log that can act like a drift fence, or near a hole/hiding place. Trap stations should be marked with a flagged pole (in grassland habitat) or flagged tree (in forest) which should be visible from one trap station to another to facilitate orientation. This prevents loss of traps and makes the trapline easy to follow and re-bait. We recommend marking each trap with a unique identifier for each trapline and station (*e.g.* A1, A2F… A15; B1, B2F… B15; etc). If two traps are placed at one station they can be distinguished by a small letter (*e.g.* A1a, A1b, A2a, A2b, etc). This is especially important if animals are brought to a central processing place to be released later at the same trap site. Marking tape and marking pen should be water resistant. Reflective station markers (*e.g.* 3M-Scotchlite™; http://solutions.3m.com or http://www.amazon.com) can be useful, if traps need to be checked at night. Marking devices should be removed after the study, unless biodegradable, non-polluting tape is used. If large herbivores

(esp. cattle) are present in the survey area, aluminium tags could be used to prevent ingestion of marking tape. In open habitats (grassland, desert) it might be necessary to tie traps to poles in order to avoid displacement by wind.

Trap inspection

Depending on trap success and habitat conditions 100-200 traps can be checked within one trap inspection. Ideally, traplines are run for a period of 3-5 days (Mühlenberg, 1993; Jones *et al.*, 1996) to reduce stress on the animals. Traps are set before sunset and checked as early as possible the following morning. All traps are then closed for the day, unless day trapping is planned. At sites where many shrews (esp. Soricinae) are expected, trap inspection at shorter intervals can prevent the animals from dying in live traps or being eaten by ants or predators in snap traps. If personnel allow it, we recommend daytime trapping at least for two days to check for diurnal species. Depending on weather conditions (*e.g.* heat) during the day, trap inspection at short intervals should be considered. Traps are baited the first day and as necessary re-baited the following days.

2.2.3. Animal handling

Most animals trapped in box (live-) traps will be alive, and a decision has to be made if a particular animal will be released after treatment or if it will be kept as a voucher specimen. Before starting a capture programme the risks of disease transmission from wildlife species (see Section 4) should be assessed. As a general precaution we recommend that the investigator wear sturdy protective gloves for handling live animals and disposable laboratory gloves during processing of dead animals. In regions with specific risks (*e.g.* hantavirus, Lassa fever) a mask or full protective gear is recommended (Mills *et al.*, 1995).

Voucher specimens

If and how many voucher specimens are to be taken from each inventory site depends on the study objectives, and also on the particular regulations and permit specifications of each site and country. Many small mammal species can not confidently be identified in the field. This can be particularly problematic for shrews. Sometimes researchers should even consider taking a larger series of hard to identify sympatric taxa. There may be a diversity of colour morphs or other phenotypically unique forms present in an area. The most interesting aspects of small mammal biology and diversity are often easily overlooked in the field. In general, we recommend keeping at least one adult male and one adult female per species from each inventory site. After the euthanasia processes (see Section 3), the animal should be accurately measured (see Section 2.2.4), prepared for preservation (see Section 3), and have tissue samples taken (Chapter 7). Finally, even when live traps are used, there is almost always some mortality. Ethically speaking, animals which die in the course of a study belong in a collection.

Animal release

The aim, when handling the animals, is to obtain the necessary information rapidly, without undue stress or injury to either the animal or the researcher. First the trapped animal is transferred from the trap into a clear, strong plastic (size 3 litres) or cloth bag. For this the door end of the trap is inserted into the opening of the bag, the door pushed open through the bag fabric, and the animal is shaken into the bag. Take care to prevent a gap between the bag and the trap through which the animal can escape. Animal and bag are weighed together with an accuracy of 0.5-1.0 g and the bag weight is substracted. Bags used need to be re-weighed frequently because of moisture and debris (bait, faeces) from the traps altering their weights. Spring balances (Pesola, etc.) of different weight classes (30 g, 100 g, 300 g) should be available depending on the size range of species captured.

Preliminary species identification can be done while the animal is held in the plastic bag until processed. In order to establish the best field identification, some body measurements besides the weight (tail, hind foot, and ear length) are taken. If more time for identification is needed the animal can temporarily be kept in a cage for observation. Photos as references can be useful. If identification remains uncertain then a representative individual should be taken as a voucher specimen.

There are two ways to handle live animals, and in our view the second option is less stressful for the animal and the researcher, and especially recommended for use by inexperienced persons.

- Grasping the animals by the nape of the neck is described by Jones *et al.* (1996). Therefore the animal is initially grasped through the bag and then bag is peeled back. After placing the animal on a flat surface, the investigator positions his/her thumb and forefinger on each side of the neck, against the back of the skull, squeezes, and pulls back, so that the fingers close only on the skin. Firmly grasping all the loose skin across the upper back, especially the skin behind the neck restricts the movement of the animal's head and allows the researcher to lift the animal and turn it to view the ventral surface for determination of sex and reproductive conditions. Several species have loose skin and cannot be grasped in this way. Likewise, holding the animal by the tail should be avoided.

- A tube of acrylic glass of an adequate calibre, both sides open, one side closed with cotton batting, is placed into the bag. The animal is then gently guided into the tube held upright and in this way calmed for further treatment. Hind feet and tail should be positioned outside the tube, and the cotton wool prevents animal's movement too far into the tube, but allows it to breathe. One finger of the investigator is always placed to prevent the animal from escaping backwards. Tubes (5-6 pieces) in different sizes (15-50 mm diameter, length 15-25 cm, Fig. 3) should be available. Avoid holding tubes with animals for extended periods to prevent overheating inside.

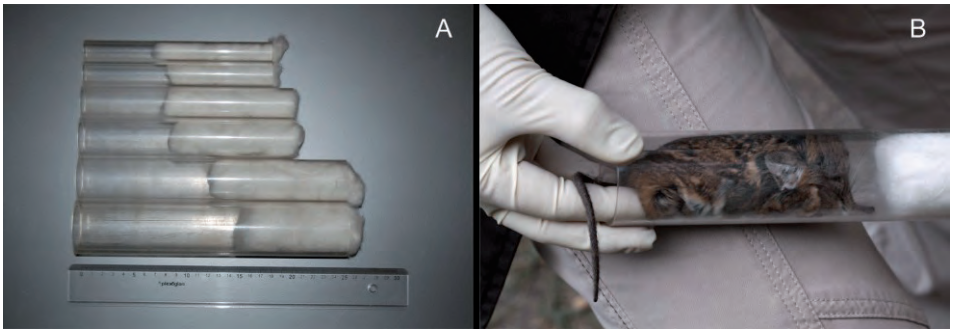


Fig. 3. A. Tubes of acrylic glass in different size; B. example of usage. (Photos by Anke Hoffmann).

After restraining the animal in this way, sex and reproductive status can be recorded and selected body measurements can be taken (see Section 2.2.4) with a calliper or ruler. If needed a tissue sample for DNA analysis can be taken from live animals (see Section 3; Chapter 7). After all data has been collected the animal should be released at the site of capture. The handling procedure usually lasts 5-10 min per animal, depending on the researcher's experience and on whether marking or parasite sampling is carried out.

Marking of animals

Released animals should be marked to avoid re-counting and re-measuring the same individuals. Marking can be done by different methods: permanent markers (tattooing, toe clipping, ear punching), or temporary markers (paints, powders) (Rudran & Kunz, 1996). When selecting an appropriate marking technique for the survey one should consider the need for individual identification, the period for which the mark should be visible, and number of animals which should be marked. For an inventory temporary markers should be sufficient and should be easy to apply. Therefore we recommend easy techniques such as hair-cutting or nail-clipping. For nail-clipping the same clipping pattern as for toe-clipping can be used (cf. Twigg, 1975, 1978). As toes are needed for pawing and personal hygiene not more than two nails per foot should be cut. Hair trimming can be applied on the back, for which the back is divided into sectors (cf. Twigg, 1978; Gurnell & Flowerdew, 2006). Both markings can be applied while the animal is in the tube (Second option above).

Sampling of parasites

Captured animals can be sampled for ectoparasites. Ticks, lice and parasitic flies can be removed from the fur and preserved in ethanol. Fleas can be collected after they jump off or have been brushed off a voucher specimen that has been euthanized inside a clean plastic bag or other closed container. It is important to keep detailed notes and cross-reference host numbers on parasite vials, field data sheets and/or field catalogue. For more details on parasite collecting see Gardner (1996).

2.2.4. Primary recording data

For each individual, sex and reproductive status should be recorded. Moreover body mass and selected body measurements (tail, hind foot, ear) should be taken (Fig. 4). Body measurements should have an accuracy of 0.5-1.0 mm. Reliable body measurements can only be taken from dead animals. Body length in particular is impossible to measure in living animals. But also the determination of sex and age is often difficult with animals to be released. Field teams should agree on whether they are using the American or European convention of standards measurements.

Sex determination

Different sexually dimorphic characters can be used to distinguish males from females, including differences in genitalia, body size, pelage, scent glands, and behaviour. Accurate sexing requires some knowledge of the natural history and morphology of individual species (Kunz *et al.*, 1996c). Primarily males are distinguished by possessing testes and a penis, females by the presence of a vaginal opening and nipples, but the visibility and spacing of the genitalia depends on age, reproductive condition and taxon. For example in many rodents the clitoris superficially resembles the male urinary papilla, but the anal-genital distance is diagnostic, typically being shorter in females than in males. Males with scrotal testes (sometimes only during the breeding season) are easy to identify, but males with non-scrotal (inguinal) testes are common, especially in Soricomorpha (shrews, moles, solenodons). The penis in some species may be retracted into a cloaca (Soricidae) and there may be other anatomical challenges like the pseudo-cloaca in *Ochotona.* Female reproductive activity is represented by gestation and lactation (enlarged nipples). The external condition of the vagina can indicate the reproductively activity in females as well, *e.g.* due to a perforated vagina or the presence of a vaginal plug (Kunz *et al*., 1996c).

Age categories

Age categories for mammals generally are listed in Kunz *et al.* (1996c). A combination of body measurements and reproductive criteria offers the best means to determine the age of small mammals in the field. Cranial and dental characteristics are valuable for an accurate age estimation done in the lab (Morris, 1972; Pucek & Lowe, 1975). For fieldwork we generally distinguish just between three age classes:

- ***Juvenile:*** A small young animal in grey and soft juvenile pelage, smaller than a subadult and not sexually mature.
- ***Subadult:*** A young animal that is not fully grown and often not in fully adult pelage. May or may not be sexually mature.
- ***Adult:*** A fully grown animal in adult pelage that is sexually mature.

Body measurements

Please be aware when taking body measurements that the European convention is different from the American one, this concerns in particular the hind foot length and the total length.

- ***Body Mass*** (BM) is measured to the closest gram by using a spring balance (see subchapter handling) for living animals, for dead animals a digital scale can also be used.
- ***Total Length*** (ToL) is the distance from the tip of the nose to the end of the fleshy part of the tail not including the tuft of hair at tail's end. Lay the voucher specimen on its back on a ruler, grasp head and tail to straighten the body and take the measurement (American convention).
- ***Tail Length*** (TL) is the distance from the base of the tail (after the anus) to the tip of the tail. Do not include the tuft of hair at the very end.
- ***Head-Body Length*** (HBL) is obtained by subtracting TL from ToL.

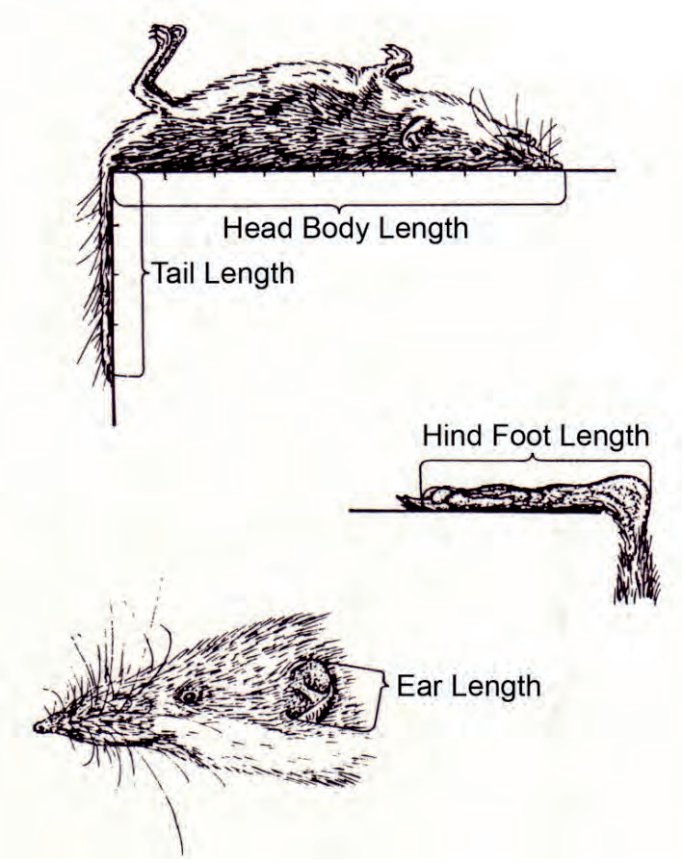


Fig. 4. Measurements of a small mammal (example: shrew). From Boye (1994), modified.

- ***Hind Foot Length*** (HFL) is the distance from the back of the heel to the end of the fleshy part of the longest toe (*s.u.* = *sine unguis*) or to the end of the largest claw (*c.u.* = *cum unguis*; American convention). Provide both measurements, when in doubt.
- ***Ear Length*** (EL) is the distance from the bottom of the notch to the furthest edge of the pinna. Before measuring grasp the ear and briefly stretch it out and release it. Hairs or tufts at the tip of the ear should not be included in the measurement.

A template data sheet for recording capture and habitat data can be designed and photocopied prior to the survey. Relevant recording data elements are listed in Appendix 1, but the selection may vary by study.

2.2.5. Workflow and personnel

We recommend a trapping period of at least 3 trap-nights with an array of about 100-150 traps and a team size of 2-3 persons (researcher, assistants) or 3-4 persons (2 researchers, 1-2 assistants) if more than 200 traps are used or bat work is planned during the night. Considering the time for set-up and removal of traplines two additional days should be scheduled. Traps can be carried to the trapline in a back-pack or strong cloth bags. One person can transport the traps and mark trap stations while the second person sets, baits and places the traps. Depending on the habitat and the distance to other traplines several hours should be scheduled for these procedures. In the morning after the last trap-night the traps can be collected just after or during the trap inspection.

We recommend at least a 2-day break between back-to-back trapping periods that require several days of camping at remote locations from the base camp. This time is needed for re-organisation, such as data entry, maintenance of field equipment like trap cleaning and mending of bat nets, preparation of vouchers and processing the by-catch (non mammals).

2.2.6. Habitat assessment

For understanding the interrelation between small mammal assemblages and their habitats, different environmental features should be assessed at the time of trapping. Habitats provide food supply, nesting sites and other hiding places. Vegetation cover, habitat structure and food availability (vegetation, arthropods) can be recorded along the traplines. More detailed vegetation surveys with species lists should be done by botanists (see Chapter 14). Rainfall and temperature data can be recorded in the microhabitat or requested from the nearest meteorological stations.

Habitat description

General description of the habitat near each trapline:

- Habitat type: type of forest, savannah, grassland, etc.; possibly list of dominant plant species.

- Altitude (m), exposition (geographic), georeference data.
- Distance to nearest water source (m).
- Existence of rocks, termite mounds, burrows, etc.
- Note: type of land use, availability of seeds and fruits, evidence of past fires, presence of large mammals, etc.

Microhabitat recording

Notes on the microhabitat of each captured individual can be recorded on standardized habitat data sheets. This may include estimations of percent canopy cover using a spherical densiometer (Forestry Suppliers Inc.) and estimations of ground cover types in a one-square meter area centred on each trap station. If there is more than one capture at the same trap station the recording of microhabitat data need not be repeated. Averages of these recordings can be used for a general habitat description. Recordings at each capture may include:

- Canopy cover (%).
- Distance and diameter breast height (dbh) of nearest tree.
- Percent ground cover types (in 1 m^2 centred on trap): herbs, grass or sedge, bare soil, leaf litter, rock, water.
- Vegetation height (cm) of ground cover.
- Vegetation density (using a density board).

The inventory of the microhabitat should be done without disturbance of the trapping procedure. The best time would be immediately after releasing or collecting the animal, during the non-activity phase or when the traps are closed. More elaborate microhabitat sampling schemes using 10-meter radius circular vegetation plots have been described by several authors (James & Shugart, 1970; James, 1978; Dueser & Shugart, 1978, 1979; Kitchings & Levy, 1981).

If the study requires the identification of a possible correlation between the diversity and abundance of small mammals and the quantity of epigaeic arthropods as available diet source, arthropod sampling can be done during the trapping period. Suitable pitfall methods are described in Chapters 9 & 15.

2.3. Bats

Bats are cryptic and nocturnal animals that are difficult to observe. Therefore, monitoring bat diversity can be a challenging task. In this section we review the most frequently used techniques to capture bats. For a more detailed methodological review we especially recommend the chapter “Methods of Capturing and Handling Bats” by Kunz *et al.* (2009). Here, we will provide a brief hands-on description of how to capture, handle and process bats in the field. Each technique may bear a certain bias in capture success and the combined

use of different methods should warrant the best success. Also, capturing protocols, *e.g.* time and duration of capture, used capturing devices, mist netting sites etc., should be consistent when comparing bat diversity among sites. Also, given that bats may carry various zoonotic diseases, such as rabies, bat workers should be familiar with health issues, *e.g.* all persons handling bats should be vaccinated against rabies (no exceptions allowed). Mist netting in or at the entry of caves may also expose people to inhalation of spores from *Histoplasma capsulatum*, a zoonotic fungus (Di Salvo *et al.*, 1969). As a general rule, all people involved in capturing bats should be informed about potential health risks.

2.3.1. Nets and traps

Ground-based mist nets

Mist nets set up horizontally and ground-based are the most common and most efficient devices to capture flying bats. Mist nets are made out of a mesh of fine synthetic fibres (monofilament nylon and braided nylon or Dacron polyester). For capturing bats, the net material is usually black and the strength of the net (mono- versus bifilament and thickness of the nylon) is chosen according to the size of the expected bats. In general, most people use mist nets with the following features: 50 denier, 2 ply nylon and 28 mm mesh size. If using thicker mist net material (higher denier value), the net can withstand larger bats, but the net is more easily detectable by the bats. Standard net sizes are 6 m (18´), 12 m (42´) or 18 m (60´) long and 2.1 m to 2.4 m high when set. Usually, the height is divided by several horizontal shelf strings that form 4 or 5 horizontal loose pockets, which hold the trapped bats once they bounce against the layer of net material and drop into the pockets. Each end of the shelf string has a loop of stronger string material that can be put around supporting poles. These poles, *e.g.* aluminium tent poles or bamboo culms, should be set up at a distance equal to the net length (Kunz *et al.*, 1996b). For setting up a mist net, the loops are placed around the first pole. The top loop, which is usually white or coloured, and the following loops should be attached in the right order from top to base. The first pole is tied with ropes to either vegetation (*e.g.* nearby trees) or attached to stakes put into the ground. If no tree is close to the net, two ropes or one twisted around the pole may be used to stabilize the net. With the two ends of the rope/ropes attached to a near-by tree or a stake, an angle of approximately 70° is established between the two ropes. To provide a better support, the base of poles should be pressed slightly into the ground. The net should be held with caution as it will unwrap itself when the carrier slowly walks towards the second pole. It should be taken care not to let the net touch the ground during that process as *e.g.* leaves might get entangled in the net. After placing the second set of loops around the second pole, with the white (or coloured) loop at the very top, once again ropes are used to tie the pole to trees, branches or stakes. The ropes should be tied in a way that moderate tension is inflicted on the net. In the last step, positioning the loops from top to the base of the poles should unclose the net. Once the mist net is open, the net material should form a pocket at each shelf string.

Mist nets should be closed during a break of a night capture session or when using a mist net more than one night at the same location. All debris such as leaves should be removed from the net before pushing the shelves together to close the net. The net can be furled by draping the net repeatedly around the gathered shelves and tucking the loose ends of the net pockets into the shelf strings. Gently spinning can also be used to furl a net. Several short strips of cloth or rope should be tied around the net to prevent unwinding.

For dismantling a mist net, the loops of the first pole are gathered at the top and are then removed from the pole, still maintaining the correct top to bottom order and keeping tension on the net to prevent it from touching the ground. The top loop should be used to tie the other loops before folding the net. By doing this, it is easier to maintain the top to base order when unravelling the net the next time. The loops of the second pole are removed in the same way as before. The net should be folded before storing it in a bag, preferably in a cotton bag as plastic bags restrict air circulation and therefore support fungal growth on the net material. Mist nets may become wet after rain or at high humidity. Then, nets should be dried before storing them over a prolonged period of time in bags.

To cover the sub-canopy of the forest, mist nets can also be stacked on top of one another. Freestanding poles with a rigging system (Rautenbach, 1985) optimize this system. The loops can be attached to carabiners on a hoist with strong free-standing net poles, which allows raising the net(s) high above the forest floor.

Canopy mist nets

Several previous studies have highlighted that bat assemblage composition differs between the ground and canopy level (*e.g.* Francis, 1994). Some species may not even be captured at ground level, because of their exclusive canopy lifestyle, *e.g.* molossid bats, large pteropodids or some phyllostomid bat species. Thus, vertical stratification of a bat assemblage is an important aspect when assessing local bat species diversity. In general, two methods are available for capturing bats at canopy height: either suspended horizontal mist nets as described in the previous paragraph or suspended vertical canopy mist nets. Both techniques require some training and sufficient time for preparation. To suspend a canopy mist net, it is necessary to first search for a good spot that provides (1) sufficient open space so that the net does not get entangled with twigs and branches, and (2) some large sturdy branches from which a rope can suspend the net. Horizontal canopy mist nets require two of such branches at distances larger than the length of the canopy mist net, whereas vertical canopy mist nets require only a single branch. The chosen branches should be sufficiently strong to support the weight of poles, ropes and mist nets. To hoist canopy nets to canopy height, it is first necessary to shoot a line with a lead fishing weight at its end over the branch. Use two lines to hoist a horizontal canopy net and one line for a vertical canopy nets. This is best achieved by using a slingshot (Kunz & Kurta, 1988; Nadkarni, 1988; Munn, 1991), a bow and arrow (Greenlaw & Swinebroad, 1967), a crossbow or a line-shooting gun. Since the thin line gets easily entangled during this process, it is best to use an

open-faced spinning reel that can be purchased from a fishing store. The line is then used to hoist a heavier cord (at least 10 mm in diameter and longer than twice the height of the branch) over the branch. Also, it should be noted that protective devices should be worn when using sling shoots or similar devices to prevent accidents. Alternatively, ropes can also be positioned by climbing. Vertical and horizontal canopy nets differ in some main features and in the way they are operated.

Vertical canopy nets require two branches from which the net is suspended and consequently two ropes. Once the rope is put around the branch in the canopy, one end should be attached to the top of the pole and the other end to the base of the same pole. This is repeated with the second rope and the second pole. Care should be taken that sufficiently strong knots are made to support the weight of the poles, ropes and net. Alternatively, carabiners can be permanently attached to the poles to warrant more support. Ideally, two persons are present when hoisting the canopy net by pulling the rope that is attached to the top of the pole. Caution should be taken not to stand right below the net in case the net or branches fall to the ground. Also, people should wear gloves when hoisting and manipulating the net. Once the net is positioned in the canopy, the rope should be attached to a tree trunk or some sturdy branches. Persons operating these nets should attach the rope very tightly to the vegetation structure. Afterwards, the opposite end of the rope that is attached to the base of the pole is manipulated in a way so that the vertical canopy net expands to its full size. The opposite ends of the ropes are also attached to vegetation to stabilize the canopy net. In order to put a canopy mist net down, it is necessary to first unknot both ends attached to the base of the pole. Then, the ends of the rope attached to the top of the pole are unknotted and held firmly with both hands. We recommend laying out a plastic tarp on the ground where the canopy mist net is supposed to stand on the ground to prevent leaves and debris from getting entangled in the net.

Vertical mist nets are designed for the purpose of canopy mist netting and bear the great advantage that they can be hoisted and handled by a single person (see Kunz *et al.*, 2009 for a detailed description). They are made out of the same material, but have a vertical instead of a horizontal rectangular shape. Usually, they are 6 to 9 m high and 3 to 4 m wide. Accordingly they do not have 4 to 5 horizontal shelf strings like a horizontal mist net, but 8 to 10 shelf strings. Three ropes (10 mm diameter) are required to deploy a vertical canopy net. A support rope with a length of at least twice the canopy height and a carabineer attached to its end. This support rope is put around an exposed sturdy branch as described before. A second rope of approximately the length of the pole is then attached from end to the other end of the pole. A third rope is then attached to the second rope at equal distance to the pole's ends. This third rope is guided through the carabineer of the first rope (the one suspending from the branch). Then, the support rope is pulled so that the carabineer at its end is at the desired height. Afterwards, the canopy net is hoisted by pulling the rope, which is attached to the pole rope. A fourth rope can be attached to the base pole to facility the operation of the net in case it gets entangled in branches. Again, a plastic tarp should be placed at the spot where the canopy net is put down (Fig. 5).

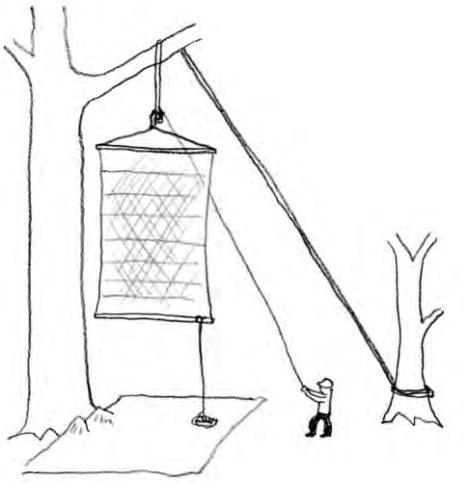


Fig. 5. Schematic view of a vertical canopy mist net (modified after Kunz *et al.*, 1988). The net is hoisted via a pulley into the canopy. A tarp is put on the floor on which the mist net can be lowered when bats are extracted from the net.

Harp traps

Harp traps have been a successful addition to the tool case of bat biologists, since bats that are never trapped with mist nets are sometimes captured with harp traps. The reason for this is that fishing lines are very difficult to detect for bats based on acoustical (or visual) cues. An additional advantage of harp traps is that bats can be more easily removed from them. Usually, harp traps consist of 2 to 4 parallel rectangular metal frames (usually 2 m x 3 m) at distances of 4 to 6 cm that each carries a layer of vertically oriented monofilament fishing lines at distances of 2-3 cm (Fig. 6).

Normally, lines of the outer layer are separated at somewhat larger distances than the inner layer (in case of three layers). Flying bats usually fall through the first layer by the momentum of their flight or manage to manoeuvre around the lines of the first layer but they will hit the second layer. Then, bats fall to the base of the harp trap into a large canvas or plastic bag. Captured bats can be easily picked out of this bag. Harp traps with four layers of lines have been successful for capturing palaeotropical insectivorous bats (Kingston *et al.*, 2003). The tension of lines, the number of line layers, and the placement of the harp trap greatly affect its capturing success.

Fig. 6. Harp trap set up in front of the exit hole of a daytime roost. Two bat species, *Noctilio albiventris* and *Molossus molossus* emerged from the roost, hit the layer of fishing lines and tumbled into the plastic bag from which they were quickly recovered and transferred to linen bag. (Photo by D.K.N. Dechmann).

2.3.2. Trapping procedure

Optimal sites for mist nets

Finding the right spot for putting up mist nets is crucial for a successful mist netting night. In general, capture success is enhanced when nets are put at natural flyways, *e.g.* at a perpendicular angle to a forest edge or across forest trails. Distinct objects such as cave entrances, buildings, rocks, water holes, etc. also present good mist netting sites. If bats pass by a certain structure on a daily basis or emerge from known roosts such as cave entrances, a mist net will yield a large number of captured bats within a short time period. It may be worth counting or estimating bats that emerge from a roost before putting up a mist net. The capture success is enhanced when several mist nets are used at the same time, preferably in a T-, Z-, Y or V pattern. Feeding sites are also suitable for capturing specific species. A fruiting or flowering tree will probably attract several bat species in the tropics or subtropics. Some bat species are attracted by artificial volatiles *e.g.* Neotropical nectar-feeding bats by Dimethyldisulphide (Helversen *et al.*, 2000) and fruit-eating bats by essential oils (Mikich *et al.*, 2003) or the pulp of fruits (Rieger & Jakob, 1988). Some species are lured into nets by playback of their prey, conspecific social or echolocation calls.

Optimal sites for harp traps

Harp traps are most efficient when set up at natural flyways of bats (see above). Since bats can be removed from harp traps at a faster rate than from mist nets, harp traps should be chosen, when large numbers of bats are expected, *e.g.* in front of daytime roosts, at cave entrances, etc. Like canopy nets, harp traps can also be suspended from large trees or into a canyon.

Time of capture

The number of nets depends on the expected number of bats per mist net, the number of field workers available and the duration of mist netting. Usually capturing devices should be set up before sunset, because the 1 to 2 hours time period following sunset is often the most rewarding time in terms of number of bats. Mist nets and harp traps should be controlled on a regular schedule depending on the frequency of captures. In general, a net or trap should be checked at least every 15 minutes during the peak activity time after sunset. Bats will readily bite large holes into mist nets while trying to find their way out of the net. Also, bats may get severely entangled when their presence in the net is overlooked. Thus, regular visual inspection of mist nets is important.

Capture success decreases as night processes. Mist netting success also drops drastically, when mist nets or harp traps are set up at the same spot during subsequent nights. Comparative studies need to ensure that capturing effort (= total time of mist netting and total length of used mist nets) is about the same for all study areas.

2.3.3. Animal handling

Removing bats from mist nets

With some experience, most bats can be removed from a mist net within a short time period. In general, many field workers prefer to wear at least one glove to be protected while holding the bat and then use the other hand to extract it from the net. When a bat is found entangled in the net, first of all it is essential to assess from which direction it has flown into the net. As a general rule, those parts of the bat that entered the net last should be removed first. Therefore, it is important to check whether the legs of the bat can be grabbed directly. During the whole process, it is most important to take care that the bat is not injured. A bat's finger and forearm bones are particularly vulnerable to physical fraction. Difficulties may arise when bats get entangled for a prolonged period of time and when the size of finger bones and forearm matches closely with the mesh size. In that case, one should start to work first at a single wing, extracting the fingers and the forearm carefully from the net. Sometimes it helps to expand the wing moderately. Occasionally, bats get irritated or distressed during the removal process and may start to bite and emit distress calls. Bats should then be held firmly and possibly a linen bag should be put close or around the bat's head to provide something for the bat to bite. In some rare instances it might be helpful to have a pair of small scissors at hand to cut some net strands into which the bat is hopelessly entangled. Sometimes bats may bite into the net, the string or the glove. Never pull the object away from the bat, but instead blow frontally against the bat's head. Eventually the bat will let go. If this is not the case, use forceps, Q-tips or a small stick to gently open the bat's mandibles. Once bats are removed from mist nets, they can be kept in a linen or cotton bag over a short period of time. In case the bat is supposed to be released, a linen bag that is wrapped around the bat's body will also facilitate measurements and species identification.

Keeping bats temporarily

Soft linen bags are best for keeping bats temporarily. Some materials are too rough for the skin of bats, especially for the joint of the forearms, which causes irritations and may consequently lead to inflammations. Therefore, bags should have approximately thrice the size of the bat in length and width. Preferably a single bat is put into one bag. Sometimes it may be necessary to put several bats into a single bag. Then, only individuals of the same species should share the same bag. However, we advise researchers to avoid this situation as even individuals of the same species may bite each other when forced to share a bag. Bats that foraged successfully before being trapped in a net can be kept over several hours in a bag. Sometimes bats will enter torpor, *i.e.* they reduce their body temperature, when kept over a prolonged period of time. Some fruit-eating and nectar-feeding bats, in particular small ones, should be fed with diluted honey water before keeping them in a capture bag. They may also benefit from a few droplets of honey water shortly before they are released.

2.3.4. Primary recording data

Species identification

When bats are released at the site of capture it is important to identify the species in the field. Identification keys are available for some regions of the world, but not for all. Sometimes, the primary literature needs to be studied prior to the field trip. In many cases it is essential to bring copies of the primary and secondary literature to the field. If animals are collected as museum specimens, identification may be postponed until it is routinely done at the museum. However, to prevent collection of a superfluous large number of specimens of the same species, we advise a rough identification of all captured individuals beforehand.

Sex determination

For the determination of sex, it is important to examine primary and secondary sexual characteristics, most importantly the genitalia. In some species, both sexes have well-developed nipples, females may have penis-like clitoris or males may have minute penis. Therefore, a combination of different traits is most useful for assessing the sex of a bat.

The reproductive status of females is checked by gentle palpation of the abdomen. If the reproductive status is important for the study, the reproductive tract has to be examined after dissection and the size of the fetus (if present) has to be measured. The examination of the nipples will provide information about whether a female has lactated or not (Racey, 1988). If the nipples are enlarged and keratinized, the female is lactating or has lactated very recently. Lactating bats should never be kept over a prolonged period of time and be released as soon as possible after processing. In lactating females the area surrounding the nipples is usually lacking fur.

Males should be checked for inguinal (abdominal) or scrotal testes and epididymis. Frequently, testes ascend into the abdominal cavity. To verify the correct sex, testes can be forced to descend by gently pushing the abdomen. We encourage to record scrotal males and whether a female is lactating or not.

Age categories

Age is categorized in bats as juvenile, subadult and adult.

- ***Juveniles*** are generally defined as non-volant young individuals that are smaller and weigh less than adult individuals. They are often captured together with their mother. The epiphyses of their bones are not fused, yet, *i.e.* there is a light area of a few mm close to the joints of the finger bones (best examined in the finger bones by shining with a torch light through the wing from underneath the bat), the pelage is often grayer than the adult fur and they have deciduous teeth.

- ***Subadults*** are volant and fully-grown, but still show the unfused epiphyses (Anthony, 1988).
- ***Adults*** show mature size, fused epiphyses, pelage and are often reproductively active, *i.e.* males may have large (scrotal or abdominal) testes and females may be either pregnant or lactating.

Body measurements

The standard measurements for bats include head-body length, tail length, hind foot length, ear length, forearm length, and body mass (Handley, 1988).

- ***Body Mass*** (BM) is recorded in grams with small spring-scales available in a variety of sizes from 10 g up to 2000 g (*e.g.* Pesola) and used accordingly to the animals' size. Especially for small bats, it should be checked whether the bat had ingested food before the capture (a bat caught very early in the night might not have had the chance to ingest a measureable amount of food). Keeping the bat for a period of time and recording the weight after excretion might provide a relatively exact weight of small bats (important for bats with body weights of less than 7 g).
- ***Head-Body Length*** (HBL) of a bat is the distance between the back of the bat's pelvis and the tip of the snout (European convention) or the distance between the last caudal vertebra (for bats with tails) and the tip of the snout (American convention). It should be measured to the nearest 1 mm.
- ***Tail Length*** (TL) is the distance from the base of the pelvis to the tip of the tail. The zero end of the ruler is placed at the base of the tail and the tail is straightened on the ruler and measured to the nearest 1 mm.
- ***Hind Foot Length*** (HFL) is the distance from the base of the calcar or the calcaneum in bats lacking a calcar respectively to the tip of the longest toe (tip of the claw for American convention). The foot is flattened on a ruler and the length is recorded to the nearest 1 mm.
- ***Ear Length*** (EL) is measured with a ruler placed gently in the notch at the base of the ear. The distance between the base and the tip of the pinna should be measured to the nearest 0.5 mm. In presence of a tragus, form and length are recorded.
- ***Forearm Length*** (FA) is defined as the distance from the elbow (tip of the ulnar olecranon process) to the wrist. To measure the forearm, the wing has to be folded. A ruler can be used, but a sliding calliper is more convenient to record to the nearest 0.5 mm.

Apart from these 5 standard field measurements, other data may also be relevant for species identification, *e.g.* the colour of the fur and presence/ absence of colour patterns (*e.g.* epaulettes, stripes; Handley, 1988).

Other field notes

For each ***capturing site***, the GPS and location data (*e.g.* distances to road, river, building, etc.) should be gathered. Additionally, it is important to write

down the number and types of nets used. A brief description of the vegetation and type of habitat (*e.g.* gallery forest, primary forest, savannah) will help future data analysis. In some instances, it may also be helpful to draw a map of the capturing sites. If possible, meteorological data from a close-by weather station should be noted. As a general recommendation, the season (rainy or dry season), the cloud cover and the moon phase should be recorded in the field book.

For each ***captured bat*** it is important to note the site of the net, the net number and type and the height above ground (net shelf). It is essential to record the time of capture and if applicable the type and location of the roost. In addition, notes should be made regarding the number of ectoparasites and whether the captured bat was a recapture. All samples (*e.g.* faecal, pollen, blood, tissue samples) taken from the specimen in the field should be labelled and identification numbers should be added to the field note book. Appendix 2 provides a template data sheet.

2.3.5. Acoustic techniques

Acoustic sampling of bats provides the inventory with important additional information. Bats, which routinely fly beyond the reach of nets and traps, can be sampled. Many different bat detectors are used to identify bat species without the necessity to capture the animals. For more detailed information see Chapter 5.

2.3.6. Workflow and personnel

Mist nets should be set up before sunset. Care should be taken not to open the nets too early, because birds could get trapped as well. We recommend setting up a work station where people can work nearby using chairs and a table when dealing with several mist nets at the same time and when many bats are expected. Once mist nets are opened, they should be checked at regular intervals depending on the frequency of bat captures.

If many different samples (*e.g.* ectoparasites, wing punches, blood samples) are planned to be collected from each captured bat and a high number of bats are expected at one site, it might be helpful to divide the work. One person should check the nets and traps frequently and store the captured bats in bat bags and provide each bag with a small sheet of paper with notes (net number and shelf, time of capture). A second person measures and identifies the bats, while a third person is taking the samples and releases the animals (or keeps the animals in case of specimen preservation).

3. Preservation and DNA sampling

Depending on the project objectives, the collection and preservation of whole voucher specimens, or taking DNA tissue samples from live animals are the best way to identify and document small mammals and bats. For any voucher

preservation, collection and import and export permits need to be obtained well in advance of the field work and special regulations regarding the presence of threatened or endangered species should be known (see also Chapter 3).

For the collection of specimens, the following five conditions should be met:

- Obtain the appropriate training (and practice) in the preparation and preservation of vouchers along with all health & safety precautions and equipment.
- Obtain research methodology clearance from your institutional animal care and use committee (IACUC in the US). See the recent review by Gannon *et al.* (2007) for the US.
- Arrange with a well-curated and officially recognized collection for accession of your specimens upon return from the field. No vouchers should be held in "private" or "personal" collections for extended periods.
- Obtain all necessary collecting and export permits from the host country or state and customs and other import permits from the country where the vouchers are to be housed (USFWS form 3-177 in the US).
- Keep meticulous records. Attach basic field data and/or a unique number to each specimen and cross-reference it in your field catalogue or field journal (Yates *et al.*, 1996). Do not rely solely on electronic records!

The field methods used to kill animals should be quick and as painless as possible for the animal. Humane methods for euthanizing small mammals in the field include the use of inhalants like Isofluran and cervical dislocation (Simmons & Voss, 2009). Lethal injection is another method, but it requires veterinary training. We highly recommend following regulations of the particular country, *e.g.* Veterinary Medical Associations.

Specimens to be kept as vouchers can be killed in a large, tightly closable container (large wide-mouth lab jars, large pickle jar, some plastic buckets with lids) in which a cotton swab soaked with an inhalation anaesthetic such as Isofluran or Enfluran has been placed. The animal should be left in the container for about 20-30 min. To avoid needless stress for larger animal we suggest placing the animal in the trap together with the anaesthetic in a tightly sealing durable plastic bag. Anaesthetics can be difficult to obtain in tropical countries (contact the country's chief veterinarian office and/or hospital medical supply companies). Most anaesthetics are controlled substances for airline travel and can only be transported by air with special permits and specially labelled packaging. See the 2007 report of the AVMA (American Veterinary Medical Association) Panel on Euthanasia for more details (AVMA, 2007). Gannon *et al.* (2007) also recommend quick mechanical methods like cervical dislocation for mammals of small body size, instead of the extra steps of sedation and anaesthesia that might only add distress to the animals. Field workers should receive the appropriate training and permits for all of these methods!

Depending on the specific study goal, different ways of preservation are possible for voucher specimens: museum dry mounts with skull or skeleton or complete liquid preservation.

Irrespective of the type of preservation, standard measurements should be taken from all specimens before fixation and dead animals should be processed as quickly as possible. All specimens should be tagged. Tags on dry specimens should note the (1) collection date, (2) capture locality, (3) collector, (4) field measurements (Yates *et al.*, 1996). Tags on fluid preserved specimens should only note the specimen's sex, the collector's initials and the field catalogue number.

Fluid preservation

Fluid preservation is increasingly being used over making dry mounts to save field time for other activities. It is the preferred method for bats to preserve important diagnostic facial features (nose-leaves, etc.). DNA tissue samples from internal organs *(e.g.* liver, spleen, kidneys) are taken before fluid preservation. The skull can be removed immediately or later in the museum. Fur colour should be recorded as exactly as possible before preparing the specimen because it will fade over time in fluid preservation (Simmons & Voss, 2009). Usually specimens are now fixed directly in 75% ethanol without intermediate fixing in formalin (Handley, 1988). In most museum collections the storage media are ethyl alcohol or isopropyl alcohol. If the specimen has not been opened to extract DNA tissue samples or to remove internal organs a certain amount of the storage media should be injected into the specimen's abdomen using a conventional syringe and needle. This is particularly important in large specimens because fermentation of ingesta in the digestive tract will damage the abdominal tissue. All specimens should be preserved in containers that are filled with sufficient amounts of fluid and all containers should be tightly sealed. No pinning or other preparations are required, except for a bit of manual manipulation of the carcass to straighten it out – in cases where the specimen has died in a contorted or curled-up position (Griffin & Kolberg, 2004).

Fluid-submerged labels should be of 100% rag paper and labelled with permanent ink (*e.g.* Pelican fine drawing ink or similar). Test permanence of inks/markers before leaving for the field! Attach the label to the right hind foot of specimens with 100% cotton string (Yates *et al.*, 1996). For field transportation fluid-preserved specimens remain submerged in ethanol in a tightly sealed container carried upright (*e.g.* wide-mouth barrel normally used for water sports: http://www.curtec.com; available from *e.g.* http://www.globetrotter.de).

For overseas transportation fluid preserved specimens can be temporarily preserved by wrapping them in several layers of cotton cheesecloth soaked in ethanol (moist but not dripping wet!) and packed in a triple layer of zip loc plastic bags inside a sealed container. In this way they can be safely transported for up to three days. However, specimens should not be preserved in such a way over a prolonged period of time.

Dry skins

Prepared dry skins have the advantage of preserving fur colour variations and of being relatively easy to transport, store, and manage long-term in collections,

as they do not require special fire-safe storage facilities and regular fluid level controls.

In museum dry mounts of small mammals cotton-filled and subsequently thoroughly dried skins of the animals are preserved with tail and feet attached. The skull or entire skeleton are usually dried or temporarily stored in ethanol and later cleaned with the help of dermestid beetle larvae before they are rinsed and dried again for the collection. We do not provide guidelines for making dry mounts here but refer readers to various detailed and well-illustrated sources (Hall, 1962; Setzer, 1968; Nagorsen & Peterson, 1980; Griffin & Kolberg, 2004) and the abbreviated recommendations in Yates *et al.* (1996). All of these sources also discuss standard methods of field catalogue and journal keeping and appropriate tagging of specimens (Fig. 7).

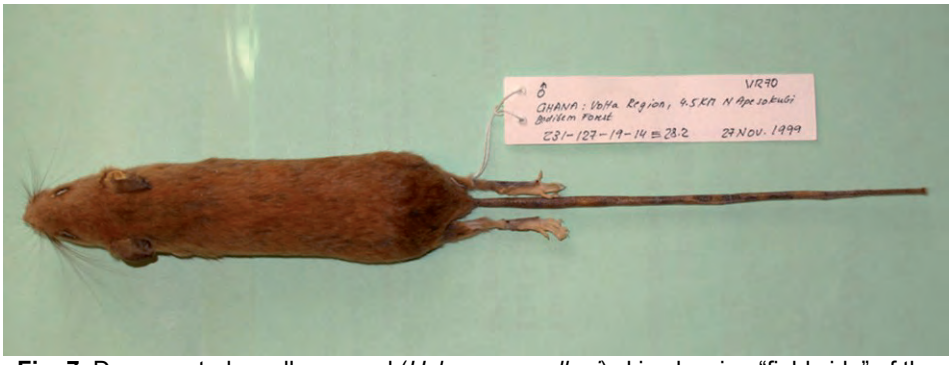


Fig. 7. Dry-mounted small mammal (*Hylomyscus alleni*) skin showing "field side" of the specimen tag with sex, field (collector's) number, locality, field measurements (total length-tail-hind foot-ear-weight) and date. (Photo by Jan Decher)

Dry mounted specimens should remain pinned on a foam board or Styrofoam sheets for several days. In the field these sheets can be cut to fit in shallow plastic ("Tupperware") containers where the specimens can be stored safely from ants and humidity at night or during rain when not being air-dried. A desiccant (Silicagel-type), which can be recharged by heating over a small fire or camp stove should be placed in cloth bags inside the specimen containers at night and during transport. Air-drying skulls or skeletons should be hung from wire rings to keep away ants or other predators and/or lay them in a little screen cage to protect them from insects. For overseas transport, specimens should be un-pinned from the foam sheets and packed in layers of cotton inside the plastic tubs, which can then placed in expedition boxes or duffle bags padded with clothes. Skulls can be packed with fluid-preserved materials. If specimens cannot be prepared immediately post mortem, they should be stored in ethanol or in plastic bags (to reduce dehydration) and kept frozen until further treatment.

DNA samples

Species identification or the verification of the morphologically identified species in the field can be achieved by DNA analysis. In many cases for the sampling of DNA tissues the animals do not need to be killed.

Taking wing biopsy punches or small amounts of blood are the most common DNA sampling methods for bats. Blood can be obtained from venous puncture of the antebrachial vein running along the anterior edge of the antebrachium or of the major vein in the interfemoral membrane (Kunz & Kurta, 1988; Watt & Fenton, 1995). Small amounts of blood can be collected in heparinised hematocrit tubes and larger samples should be collected by using heparinised syringes (Dessauer *et al.*, 1990, 1996; Prendini *et al.*, 2002). If the project design aims to quantify blood parasites or other pathogens, blood should be collected on a filter paper and/or prepared as a blood smear. Blood smears are usually fixed in methanol and air-dried.

Tissue samples for genetic analyses can be collected from bats by puncturing the wing membrane (chiropatagium) or the tail membrane (uropatagium) using biopsy punches (Worthington-Wilmer & Barratt, 1996). The chiropatagium is easy to access, is less vascularized and bleeds less compared to the tail membrane (uropatagium). A 3 mm diameter biopsy punch will yield sufficient DNA for future analysis. When taking biopsy samples from the wing membrane, care should be taken not to cause damage to larger veins. The results of Faure *et al.* (2009) show that tail wounds healed significantly faster than wing wounds and more DNA from tail biopsies could be extracted than from wing biopsies of the same size. They recommend that tissue biopsy for molecular analyses in bats should be taken from the tail membrane. Biopsies of the wing membrane are useful for marking associated with recapture programmes, because the wound and scar will persist longer (Faure *et al.*, 2009). The hole, which is left in the membrane after puncture, will close and heal within 2 to 4 weeks. If dry mounted or fluid specimens are being collected, small DNA tissues from internal organs (liver, kidney, spleen) can be sampled, before these organs are removed from the specimens.

Tissue samples from live small terrestrial mammals can be collected by tail-clipping or ear punching. The tail-clipping method implies the amputation of a small portion (1-2 mm) of the distal tail using sharp scissors. The ear punch method involves punching a hole or making a notch in the ear. Both methods do not require the use of anaesthesia or analgetics. In case toe-clipping is applied as a marking method (see Section 2.2.3) the amputated phalange can be used for genetic analyses. The tissue is immediately transferred in a vial with preserving solution (see Chapter 7). Afterwards the scissors and biopsies punch should be disinfected by dipping the tool into 95 % ethanol and burning the liquid off with a lighter flame.

The best preservation of fresh tissue samples can be achieved by freezing the samples using either dry ice or liquid nitrogen (Prendini *et al.*, 2002) or preserving in a lysis buffer (Longmire *et al.*, 1997). Placing the tissue sample directly in 95-100% ethanol in leak-proof 5 ml cintillation vials (or similar small plastic containers) will be more feasible in most situations (Kilpatrick, 2002). For

long-term preservation all tissues should be kept frozen (methods for collecting, storing, and archiving tissue samples: Dessauer *et al.*, 1990, 1996; Longmire *et al.*, 1997; Kilpatrick, 2002; Prendini *et al.*, 2002). For more detailed DNA/tissue collection techniques see Chapter 7. The collection of fur, faecal and pollen samples might be useful for a variety of further studies and we recommend the book edited by Kunz & Parsons (2009) as a reference.

4. Safety precautions

Handling wild animals can always include the possibility of exposure to zoonotic diseases (Childs *et al.*, 1995; Gage *et al.*, 1995; Kunz *et al.*, 1996a; Chomel *et al.*, 2007). Some of these pathogens may have limited health risks others can lead to fatal diseases (*i.e.* rabies). This short section discusses some issues regarding the reduction of health risk during field work. Zoonotic pathogens are transmitted by various routes. Beside the well-known "rabid bite", where the rabies virus is transmitted into the wound via saliva of an infected animal, all other body fluids can also contain infectious agents. Urine from rodents, for example, may be a source of hantavirus or *Leptospira* spp. (Levett, 2001; Fulhorst *et al.*, 2007; Machado *et al.*, 2009). Even if mice are not handled directly, urine may have contaminated traps or the surrounding soil. Aeroionisation is the typical way of contracting a hantavirus infection (Machado *et al.*, 2009; Olsson *et al.*, 2009). Faeces, blood, and fur can also contain infectious pathogens. Wearing disposable gloves should become routine habit when working in direct contact with wild animals. With some larger or more aggressive species leather gloves are a good protection against bites and scratches. When gloves become soiled by the animal's excretions, they can be disinfected with a spray solution, which needs to be designed to eliminate viruses, bacteria and fungi on most material surfaces (*e.g.* Pursept-A Xpress® (Merz)) at the end of a day's/night's field work. The manufacturer's instructions should be checked beforehand as not all disinfectants will destroy every pathogen. Many disinfectants are only designed to destroy bacteria and do not contain protective remedies for certain viruses or protozoa. A hypochlorite solution (household bleach) in a 1:10 dilution can also be used. If using a spray, avoid inhaling the aerosol, for example outdoors by monitoring the wind direction. Spray solution can also be used to wipe off all equipment (including traps) and sample containers. For skin disinfection, products are available that are specially made for this particular purpose (for example: Virusept Manorapid Synergy® (Merz)), which are less aggressive and also contain some skin care ingredients.

Another important pathogen vector is dust. Face masks can prevent inhaling aerosols and/or light particulate matter. Surgical masks may be a first precaution, but they cannot be considered pathogen safe as they do not seal. A safe mask needs to cover mouth and nose without any gap and should remain so for some time. However, in a hot and humid environment the mask's fabric easily gets soaked with moisture allowing particles to enter and should be replaced in time. So-called FFP3 masks (for example manufactured by 3MTR) feature a small breathing filter to allow air to enter at a lower point of resistance simultaneously keeping the rim of the mask better attached to the skin. As

peoples' faces are differently shaped it is advisable to try out differently shaped/sized masks before setting off. Additionally, protective eye gear might have to be included for field work, particularly if there is a possibility to be exposed to contaminated material dropping from ceiling inside caves, etc., or exposure to blood or urine. Mucous membranes can serve as contact points for many pathogens and the eyes are least protected by the immune system. Overalls protect and allow discriminating between contaminated working and everyday clothing.

Carcass dissection increases the risk of exposure to zoonotic pathogens. Beside the described personal protection, dissection should be performed as safe and clean as possible. Covering the worktop with a paper towel for each animal will give cleanliness. A separate disposal bag should be kept ready to collect soiled gloves, paper towels, etc. Sharp items like scalpel blades must be stored in an unbreakable container. In case of accidental cuts sufficient amounts of blood should be pressed out of the wound and the lesion cleaned from pathogenic agents. The wound can be washed with mineral water or safe drinking water. Afterwards a disinfectant like Povidone iodine (spray or ointment) should be applied onto the wound and covered with plaster or clean bandages. If immediate medical support is far away, the body temperature should be monitored to detect an infection, which should be medically treated with antibiotics or likewise as soon as possible.

Above all, it is important to become familiar with zoonotic pathogens that can occur in certain animal species and a particular geographical area, during the planning phase. Medical advice should be sought about vaccinations and a well-equipped first-aid kit, including medication, should be planned well in advance. All scientists who are working with bats should be vaccinated against rabies (Rupprecht *et al.*, 2008) as well as travelling to countries with high rabies prevalence should imply rabies vaccination (Briggs & Hanlon, 2007). Vaccination against tetanus should be considered obligatory for anybody working in the field. To list all zoonotic agents would exceed the given space, but the following website (http://www.merckvetmanual.com/mvm/htm/bc/tzns01.htm) provides a summary of pathogens, their animal source and means of transmission. As the distribution of diseases varies by the different geographic regions information on specific pathogens can also be retrieved from web pages of OIE, WHO or CDC.

Further information about disease risks for mammalogists can also be found in the following publications: Cox (1979); Krebs *et al.* (1995); Mills *et al.* (1995); Kunz *et al.* (1996a); Hafner (2007).

5. Practical notes

5.1. Checklist "Before launching"

Before launching a mammal biodiversity inventory, the investigator must clearly define the objective(s) of the study. The objective(s) guide the survey through all stages of planning and execution (Rudran & Foster, 1996). Fund raising, establishing contacts with other experts, review of scientific literature, purchase

of equipment, recruitment of personnel and organisation of the travel itself (flight, visa, permits, transport of equipment, vaccinations, etc.) usually need more time then estimated in the beginning, according to experience.

The first step in preparing for a survey is to review the scientific literature for mammal studies conducted in the project area, at nearby sites or in comparable habitats in the region. The information obtained is used to compile a preliminary list of species that may be encountered at the study site. Identification keys relevant to the study area and other guide books should be obtained. For example, in the case of West Africa, the only published field key for shrews is The Shrews of Nigeria (Hutterer & Happold, 1988) and for rodents we still frequently use The Mammals of Africa, an identification manual (Meester & Setzer, 1971). If no identification keys are available for the specific study area, a preliminary survey and/or visits to museum mammal collections to become familiar with the species which might be expected, should be considered. Knowledge of the natural history (physiology, behaviour, distribution) of the target species is important for choosing the right techniques. The choice of appropriate techniques depends on the available budget and on the specifics of the field situation. Purchase of equipment and recruitment of personnel should commence as their need is really identified.

During the planning phase it is important to contact other experts and/or project coordinators to gain access to information about the region, habitat and fauna. Information about on-site logistics, *e.g.* accessibility of field sites (foot/vehicle), storage options for vouchers (esp. in the tropics), lab space (if needed), availability of drinking water, medical care, maps, etc. are helpful for planning. Please inform and prepare yourself also about human health concerns and disease risks. It might also be useful to coordinate your survey with other scientists, for example with botanists, who would provide habitat descriptions.

5.2. Field equipment

A list of field equipment for an inventory of terrestrial small mammals and bats is provided in Appendix 3. It covers: trapping, netting, treatment of the animals, tissue taking in the field, specimen preservation, habitat assessment and others. This list is not exhaustive and has to be adapted to the particular inventory. The set of equipment of course depends on *e.g.* the selected methods, selected sites, local conditions and others.

5.3. Simultaneous inventory of small mammals and bats

If nocturnal bat work is planned (see Section 2.3) it can be helpful to split the team into a bat and a terrestrial small mammal group allowing the bat group to sleep in in the morning and not participate in early morning trap checks after long hours of nocturnal netting and processing of bats (Tab. 2).

	Bat Group	Small Mammal Group
0400-0600h	resting (alternative: early morning bat netting, depending on team size)	resting
0600-0900h	resting	early morning trap inspection
0900-1000h	resting / breakfast	breakfast
1000-1300h	Bat voucher processing	Small Mammal voucher processing
1300-1400h	lunch	lunch
1400-1500h	afternoon rest / field note writing	afternoon rest / field note writing
1500-1800h	re-setting of bat nets / harp trap. Mending of nets	voucher preparation followed by replacing/opening of traps
1800-1900h	dinner & opening of nets	dinner
1900-2300h	bat netting	assist with bat netting, also nocturnal surveys for galagos, civets, pottos etc. with flash light and camera / tape recorder
2300-0100h	bat netting	resting
0100-0400h	resting	resting

Table 2. "Idealized" 2-group field team schedule for bat and small mammal work.

5.4. By-catch

Quite frequently non-target species are captured during small mammal and bat surveys. Apart from non-target mammal species a diverse suite of species belonging to invertebrates (insects, snails, etc.) and other vertebrates, *e.g.* lizards, snakes, birds are sometimes captured coincidentally.

See the corresponding Chapters in this manual for handling/preservation of these species.

- Nocturnal/crepuscular birds in mist nets; during night and day birds in tomahawk traps, large Sherman traps, snap traps.
- Snakes, lizards, amphibians in Pitfall traps, Sherman traps, Snap traps.
- Insects in mist nets (*e.g.* Coleoptera, Lepidoptera), Sherman traps, Pitfall traps.

6. References

AMERICAN VETERINARY MEDICAL ASSOCIATION. 2007. AVMA Guidelines on Euthanasia. *Journal of the American Veterinary Medical Association* 218: 669-696.

ANADU, P.A., ELAMAH, P.O. & OATES J.F. 1988. The bushmeat trade in southwestern Nigeria: a case study. *Human Ecology* 16: 199-208.

ANDRZEJEWSKI, R., FEJGIN, H. & LIRO A. 1971. Trappability of trap-prone and trap-shy bank voles. *Acta Theriologica* 16: 401-412.

ANGELICI, F.M., LUISELLI, L. & POLITANO, E. 1999. Distribution and habitat of selected carnivores (Herpestidae, Mustelidae, Viverridae) in the rainforests of

southeastern Nigeria. *Zeitschrift für Säugetierkunde* 64: 116-120.

ANTHONY, E.L.P. 1988. Age determination in bats. *In*: Kunz, T.H. (Ed.). *Ecological and Behavioral Methods for the study of bats*. Smithsonian Institution Press, Washington, DC: 47-58.

ANTHONY, N.M., RIBIC, C.A. BAUTZ, R. & GARLAND JR.,T. 2005. Comparative effectiveness of Longworth and Sherman live traps. *Wildlife Society Bulletin* 33: 1018-1026.

BANG, P. & DAHLSTROM, P. 2001. *Animal tracks and signs*. Oxford University Press, UK: 264 pp.

BOURLIERE, F. 1975. Mammals, small and large: the ecological implications of size. *In*: GOLLEY, F., PETRUSEWICZ, K. & RYSZKOWSKI, L. (Eds). *Small mammals: their productivity and population dynamics*. Cambridge University Press, Cambridge, UK: 1-8.

BOWKETT, A.E., LUNT, N., ROVERO, F. & PLOWMAN, A.B. 2006. How do you monitor rare and elusive mammals? Counting duikers in Kenya, Tanzania and Zimbabwe. *In*: ZGRABCZYÑSKA, E., ĆWIERTNIA, P. & ZIOMEK, J. (Eds). *Animals, Zoos and Conservation*. Zoological Garden in Poznan, Poland: 21-28.

BOYE, P. 1994. *Heimische Säugetiere. Ein Bestimmungsschlüssel für die in der Bundesrepublik Deutschland wildlebenden Säugetiere, ausser den Ordnungen Robben, Paarhufer und Wale*. 11. Edition. Deutscher Jugendbund für Naturbeobachtungen, Hamburg: 103 pp.

BRIGGS, D. & HANLON, C.A. 2007. World Rabies Day: focussing attention on a neglected disease. *Veterinary Record* 161: 288-289.

CAUGHLEY, G. 1977. *Analysis of Vertebrate populations*. John Wiley and Sons, New York: 234 pp.

CHILDS, J.E., MILLS, J.N. & GLASS, G.E. 1995. Rodent-borne hemorrhagic fever viruses: A special risk for mammalogists? *Journal of Mammalogy* 76: 664-680.

CHOATE, J.R. 1971. Notes on geographic distribution and habitats of mammals eaten by owls in southern New England. *Kansas Academy of Science* 74: 212-216.

CHOMEL, B.B., BELOTTO, A. & MESLIN, F.-X. 2007. Wildlife, exotic pets, and emerging diseases. *Emerging Infectious Diseases* 13: 6-11.

COETZEE, C.G. 1972. The identification of Southern African small mammal remains in the owl pellets. *Cimbebasia*, *Series A* 2: 54-62.

COLE, F.R. & WILSON, D.E. 1996. Mammalian Diversity and Natural History (Chapter 2). *In*: WILSON, D.E., COLE, F.R., NICHOLS, J.D., RUDRAN, R. & FOSTER, M.S. (Eds). *Measuring and Monitoring Biological Diversity: Standard Methods for Mammals*. Smithsonian Institution Press, Washington and London: 9-39.

COLWELL, R.K., MAO, C.X. & CHANG, J. 2004. Interpolating, extrapolating, and comparing incidence-based species accumulation curves. *Ecology* 85: 2717-2727.

CORBET, G.B. & HARRIS, S. 1990. *The handbook of British mammals*. 3rd Edition. Blackwell Scientific Publications, Oxford: 588 pp.

COX, F.*E.G.* 1979. Ecological Importance of small mammals as reservoirs of disease. *In*: STODDART, D.M. (Ed.). *Ecology of small mammals*. John Wiley & Sons, New York: 213-238.

CROOKES, D.J., ANKUDEY, N. & MILNER–GULLAND, E.J. 2005. The value of a long-term bushmeat market dataset as an indicator of system dynamics. *Environmental Conservation*, 32: 333-339.

DAVIS, E.D. 1982. *CRC Handbook of Census Methods for Terrestrial Vertebrates*. CRS Press, Boca Raton, Florida: 397 pp.

DECHER, J., NORRIS, R.W. & FAHR, J. in press. Small mammal survey in the upper Seli River valley, Sierra Leone. *Mammalia*.

DELANY, M.J. 1974. *Ecology of small mammals.* Studies in Biology no. 51. Hodder & Stoughton Educational, UK: 60 pp.

DESSAUER, H.C., COLE, C.J. & HAFFNER M.S. 1990. Collection and storage of tissues. *In*: HILLIS D.M. & MORITZ, C. (Eds). *Molecular Systematics*. Sinauer, Sunderland, MA: 25-41.

DESSAUER, H.C., COLE, C.J. & HAFFNER, M.S. 1996. Collection and storage of tissues. *In*: HILLIS, D.M. MORITZ, C. & Mable B.K. (Eds). *Molecular systematics*, 2nd edition. Sinauer, Sunderland, MA: 29-47.

DI SALVO, A.F., AJELLO, L., PALMER, J.W., WINKLER, J.R. & WINKLER, W.G. 1969. Isolation of *Histoplasma capsulatum* from Arizona bats. *American Journal of Epidemiology* 89: 606-614.

DOGGART, N., PERKIN, A., KIURE, J., FJELDSA, J., POYNTON, J. & BURGESS, N. 2006. Changing places: how the results of new field work in the Rubeho Mountains influence conservation priorities in the Eastern Arc Mountains of Tanzania. *African Journal of Ecology* 44: 134-144.

DRICKAMER, L.C. 1987. Influence of time of day on captures of two species of *Peromyscus* in a New England deciduous forest. *Journal of Mammalogy* 68: 702-703.

DUESER, R.D. & SHUGART JR., H.H. 1978. Microhabitats in a forest-floor small mammal fauna. *Ecology*, 59: 89-98.

DUESER, R.D. & SHUGART JR., H.H. 1979. Niche pattern in a forest-floor small mammal fauna. *Ecology* 60: 108-118.

ELTON, C. & NICHOLSON, M. 1942. The ten-year cycle in numbers of the lynx in Canada. *Journal of Animal Ecology* 11: 215-244.

FAURE, P.A., RE, D.E. & CLARE, E.L. 2009. Wound healing in the flight membranes of Big Brown Bats. *Journal of Mammalogy* 90: 1148-1156.

FORESMAN, K.R & PEARSON, D.E. 1998. Comparison of proposed survey procedures for detection of forest carnivores. *Journal of Wildlife Management* 62: 1217-1226.

FRANCIS, C.M. 1994. Vertical stratification of fruit bats (Pteropodidae) in lowland dipterocarp rainforest in Malaysia. *Journal of Tropical Ecology* 10: 523-530

FRASER, J.L., THOMPSON, G.G. & MORO, D. 2003. Adequacy of terrestrial fauna surveys for the preparation of Environmental Impact Assessments in the mining industry of Western Australia. *Ecological Management & Restoration* 4: 187-192.

FULHORST, C.F., MILAZZO, M.L., ARMSTRONG, L.R., CHILDS, J.E., ROLLIN, P.E., KHABBAZ, R., PETERS, C.J. & KSIAZEK, T.G. 2007. Hantavirus and arenavirus antibodies in persons with occupational rodent exposure, North America. *Emerging Infectious Diseases* 13: 532-538.

GAGE, K.L., OSTFELD, R.S. & OLSON, J.G. 1995. Non-viral vector-borne zoonoses associated with mammals in the United States. *Journal of Mammalogy* 76: 695-715.

GAINES, M.S. & MCCLENAGHAN JR.,L.R. 1980. Dispersal in small mammals. *Annual Review of Ecology and Systematics*, 11: 163-196.

GANNON, W.L., SIKES, R.S. & ASM ANIMAL CARE AND USE COMMITTEE 2007. Guidelines of the American Society of Mammalogist for the use of wild animals in research. *Journal of Mammalogy* 88: 809-823. http://www.mammalogy.org/committees/commanimalcareuse/ASM%20Animal%20Guidelines.pdf

GARDNER, S.L. 1996. Field parasitology techniques for use with mammals (Appendix 6). *In*: WILSON, D.E., COLE, F.R., NICHOLS, J.D., RUDRAN, R. & FOSTER, M.S. (Eds). *Measuring and monitoring biological diversity. Standard methods for mammals*. Smithsonian Institution Press, Washington and London: 291-298.

GREENLAW, J.S. & SWINEBROAD, J. 1967. A method for constructing and erecting aerial-nets in a forest. *Bird-Banding* 38: 114-119.

GRIFFIN, M. & KOLBERG, H. 2004. *Preserving biological specimens: a guide for Namibians*. Biosystematics Working Group, National Biodiversity Programme, Windhoek, Namibia: 112 pp.

GURNELL, J. & FLOWERDEW, J.R. 2006. *Live trapping small mammals. A practical guide*. 4th Edition. The Mammal Society, London: 48 pp.

HAFNER, M.S. 2007. Field research in mammalogy: An enterprise in peril. *Journal of Mammalogy* 88: 1119 -1128.

HALL, E.R. 1962. Collecting and preparing study specimens of vertebrates. *University of Kansas Museum of Natural History, Miscellaneous Publications* 30: 1-46.

HANDLEY, C.O. JR. 1988. Specimen preparation. *In*: Kunz, T.H. (Ed). *Ecological and Behavioral Methods for the study of bats*. Smithsonian Institution Press, Washington, DC.: 437-458.

HANDLEY, C.O. & KALKO E.K.V. 1993. A short history of pitfall trapping in America, with a review of methods currently used for small mammals. *Virginia Journal of Science* 44: 19-26.

HELVERSEN, O. VON, WINKLER, L. & BESTMANN, H.J. 2000. Sulphur-containing "perfumes" attract flower-visiting bats. *Journal of Comparative Physiology A* 186: 143-153.

HOFFMANN, A. 1995. Fangeffizienz verschiedener Fallentypen für Kleinsäuger. *In*: STUBBE, M., STUBBE, A. & HEIDECKE, D. (Eds). *Methoden feldökologischer Säugetierforschung*, Martin-Luther Universität Halle-Wittenberg. Wissenschaftliche Beiträge, Band 1: 273-280.

HOFFMANN, A. 1999. Habitatnutzung und Populationsdynamik von Kleinsäugern im Grasland des Queen Elizabeth National Park, Uganda. Dissertation, Technische Universität Braunschweig, Germany: 228 pp. http://www.biblio.tu-bs.de/ediss/data/20000114a/20000114a.html.

HUTTERER, R. & HAPPOLD, D.C.D. 1983. The shrews of Nigeria. *Bonner Zoologische Monographien* 18: 1-79.

JAMES, F.C. 1978. On understanding quantitative surveys of vegetation. *American Birds* 32: 18-21.

JAMES, F.C. & SHUGART, H.H. 1970. A quantitative method of habitat description. *Audubon Field Notes* 24: 727-736.

JONES, C., MCSHEA, W.J., CONROY, M.J. & KUNZ, T.H. 1996. Capturing Mammals (Chapter 8). *In*: WILSON, D.E., COLE, F.R., NICHOLS, J.D., RUDRAN, R. & FOSTER, M.S. (Eds). *Measuring and monitoring biological diversity. Standard methods for mammals*. Smithsonian Institution Press, Washington and London: 115-155.

KALKO, E.K.V. & HANDLEY JR., C.O. 1993. Comparative studies of small mammal populations with transects of snap traps and pitfall arrays in southwestern Virginia. *Virginia Journal of Science* 44: 3-18.

KINGSTON, T., FRANCIS, C.M., AKBAR, Z. & KUNZ, T.H. 2003. Species richness in an insectivorous bat assemblage from Malaysia. *Journal of Tropical Ecology* 19: 1-12.

KILPATRICK, C.W. 2002. Non-cryogenic preservation of mammalian tissues for DNA extraction: an assessment of storage methods. *Biochemical Genetics* 40: 53-62.

KIRKLAND, G.L.J. & SHEPPARD, P.K. 1994. Proposed standard protocol for sampling small mammal communities. *In*: MERRITT, J.F., KIRKLAND, JR. G.L. & ROSE, R.K. (Eds). *Advances in the biology of shrews*. Carnegie Museum of Natural History, Special Publications, Pittsburg, Pennsylvania, 18: 277-283.

KITCHINGS, J.T. & LEVY, D.J. 1981. Habitat patterns in a small mammal community. *Journal of Mammalogy* 62: 814-820.

KREBS, C.J. 1989. *Ecological methodology*. Harper Collins Publishers, Inc., New York: xii + 654 pp.

KREBS, J.W., WILSON, M.L. & CHILDS, J.E. 1995. Rabies - epidemiology, prevention, and future research. *Journal of Mammalogy* 76: 681-694.

KUNZ, T.H. & KURTA, A. 1988. Capture methods and holding devices. *In*: KUNZ, T.H. (Ed.). *Ecological and Behavioral Methods for the study of bats.*

Smithsonian Institution Press, Washington, DC.: 1-30.

KUNZ, T.H. & PARSONS S. 2009. *Ecological and behavioral methods for the study of bats*. The Johns Hopkins University Press, Baltimore: xvii + 901 pp.

KUNZ, T.H., HODGKISON, R. & WEISE, C. 2009. Methods of capturing and handling bats. *In*: KUNZ, T.H. & Parsons, S. (Eds). *Ecological and behavioral methods for the study of bats*. The Johns Hopkins University Press, Baltimore: 3-35.

KUNZ, T.H., RUDRAN, R. & GURRI-GLASS, G. 1996a. Human Health Concerns (Appendix 2). *In*: WILSON, D.E., COLE, F.R., NICHOLS, J.D., RUDRAN, R. & FOSTER, M.S. (Eds). *Measuring and monitoring biological diversity. Standard methods for mammals*. Smithsonian Institution Press, Washington and London: 255-264.

KUNZ, T.H., TIDEMANN, C.R. & RICHARDS. G.C. 1996b. Capturing Mammals (Chapter 8): Small volant mammals. *In*: WILSON, D.E., COLE, F.R., NICHOLS, J.D., RUDRAN, R. & FOSTER, M.S. (Eds). *Measuring and monitoring biological diversity. Standard methods for mammals*. Smithsonian Institution Press, Washington and London: 122-146.

KUNZ, T.H., WEMMER, C. & HAYSSEN, V. 1996c. Sex, age, and reproductive condition of mammals (Appendix 5). *In*: WILSON, D.E., COLE, F.R., NICHOLS, J.D., RUDRAN, R. & FOSTER, M.S. (Eds). *Measuring and monitoring biological diversity. Standard methods for mammals*. Smithsonian Institution Press, Washington and London: 279-290.

LEVETT, P.N. 2001. Leptospirosis. *Clinical Microbiology Reviews* 14: 296-326.

LONGMIRE, J.L., MALTBIE, M. & BAKER, R.J. 1997. Use of "lysis buffer" in DNA isolation and its implication for museum collections. *Occasional Papers*, *Texas Tech University* 163: 1-4.

MACDONALD, D.W. & BARRETT, P. 2002. *Mammals of Europe*. Princeton University Press: 320 pp.

MACHADO, A.M., GARCIA DE FIGUEIREDO, G., SABINO DOS SANTOS JR, G. & TADEU MORAES FIGUEIREDO, L. 2009. Laboratory diagnosis of human hantavirus infection: novel insights and future potential. *Future Virology* 4: 383-389.

MALCOLM, J.R. 1991. Comparative abundance of Neotropical small mammals by trap height. *Journal of Mammalogy* 72: 188-192.

MALCOLM, J.R. 1995. Forest structure and the abundance and diversity of neotropical small mammals. *In*: LOWMAN, M.D. & NADKARNI, N.M. (Eds). *Forest canopies*. Academic Press, San Diego: 179-197.

MARTIN, R.E., DEBLASE, A.F. & PINE, R.H. 2000. A manual of mammalogy. McGaw Hill, New York: 333 pp.

MEESTER, J. & SETZER, H.W. 1971. *The mammals of Africa. An identification manual*. Smithsonian Institution Press, Washington: 15 parts, unpaginated.

MIKICH, S.B., BIANCONI, G.V., MAIA, B.H. & TEIXERIA, S.D. 2003. Attraction of the fruit-eating bat *Carollia perspicillata* to *Piper gaudichaudianum* essential oil. *Journal of Chemical Ecology* 29: 81-86.

MILLS, J.N., YATES, T.L., CHILDS, J.E., PARMENTER, R.R., KSIAZEK, T.G., ROLLIN, P.E. & PETERS, C.J. 1995. Guidelines for working with rodents potentially infected with hantavirus. *Journal of Mammalogy* 76: 716-722.

MORRIS, P. 1972. A review of mammalian age determination methods. *Mammal Review* 2: 69-104.

MÜHLENBERG, M. 1993. *Freilandökologie*. 3rd Edition. Quelle & Meyer Verlag, Heidelberg, Wiesbaden: 512 pp.

MUNN, C.A. 1991. Tropical canopy netting and shooting lines over tall trees. *Journal of Field Ornithology* 62: 454-463.

NADKARNI, H.N. 1988. Use of portable platform for observations of tropical forest canopy animals. *Biotropica* 20: 350-351.

NAGORSEN, D.W. & PETERSON, R.L. 1980. *Mammal collectors´ manual. A guide for collecting, documenting, and preparing mammal specimens for scientific research*. Royal Ontario Museum, Toronto: 79 pp.

NATIONAL GEOGRAPHIC SOCIETY 1998. Rain forest – heroes of the high frontier. VHS tape. 60 minutes.

NICOLAS, V. & COLYN, M. 2006. Relative efficiency of three types of small mammal traps in an African rainforest. *Belgian Journal of Zoology* 136: 107-111.

NICOLAS, V., BARRIÈRE, P., TAPIERO, A. & COLYN, M. 2009. Shrew species diversity and abundance in Ziama Biosphere Reserve, Guinea: comparison among primary forest, degraded forest and restoration plots. *Biodiversity and Conservation* 18: 2043-2061.

OHNESORGE, G. & SCHEIBA, B. 2007. *Tierspuren & Fährten in Feld und Wald*. Bassermann Verlag, München: 344 pp.

OLSSON, G.E., HJERTQVIST, M., LUNDKVIST, A. & HÖRNFELDT, B. 2009. Predicting high risk for human hantavirus infections, Sweden. *Emerging Infectious Diseases* 15: 104-106.

PANKAKOSKI, E. 1979. The cone trap - a useful tool for index trapping of small mammals. *Annales Zoologici Fennici* 16: 144-150.

PERRY, R.W., TAPPE, P.A., PEITZ, D.G., THILL, R.E., MELCHOIRS, M.A. & WIGLEY, T.B. 1996. A Comparison of Snap Traps for Evaluating Small Mammal Populations. *Proceedings of the Annual Conference of Southeastern Association of Fish and Wildlife Agencies* 50: 280-286.

PRENDINI L., HANNER R. & DESALLE R. 2002. Obtaining, storing and archiving specimens for molecular genetic research. *In*: DESALLE. R., GIRIBET, G. & WHEELER W. (Eds). *Methods and tools in biosciences and medicine (MTBM) techniques in molecular systematics and evolution*, Birkhauser, Basel: 176-248.

PUCEK, Z. & LOWE, V.P.W. 1975. Age criteria in small mammals. *In*: GOLLEY, F.B., PETRUSEWICZ, K. & RYSZKOWSKI, L. (Eds). *Small mammals: their productivity and population dynamics*. Cambridge University Press, Cambridge: 55-72.

RACEY, P.A. 1988. Reproductive assessment in bats. *In*: KUNZ, T.H. (Ed.). *Ecological and Behavioral Methods for the Study of Bats*. Smithsonian Institute Press, Washington, DC: 31-46.

RATHBUN, G.B. 1979. The social structure and ecology of elephant-shrews. *Zeitschrift für Tierpsychologie* 20: 1-77.

RAUTENBACH, I.L. 1985. A new technique for the efficient use of macro-mistnets. *Koedoe* 28: 1-86.

REZENDES, P. 1999. *Tracking and the art of seeing - how to read animal tracks and signs*. Harper Collins, New York: 336 pp.

RIEGER, J.F. & JAKOB, E.M. 1988. The sue of olfaction in food location by frugivorous bats. *Biotropica* 20: 161-164.

RUDRAN, R. & FOSTER, M.S. 1996. Conducting a survey to assess mammalian diversity (Chapter 5). *In*: WILSON, D.E., COLE, F.R., NICHOLS, J.D., RUDRAN, R. & FOSTER, M.S. (Eds). *Measuring and monitoring biological diversity. Standard methods for mammals*. Smithsonian Institution Press, Washington and London: 71-79.

RUDRAN, R. & KUNZ, T.H. 1996. Methods for marking mammals (Appendix 7). *In*: WILSON, D.E., COLE, F.R., NICHOLS, J.D., RUDRAN, R. & FOSTER, M.S. (Eds). *Measuring and monitoring biological diversity. Standard methods for mammals*. Smithsonian Institution Press, Washington and London: 299-310.

RUPPRECHT, C.E., BARRETT, J., BRIGGS, D., CLIQUET, F., FOOKS, A.R., LUMLERTDACHA, B., MESLIN, F.X., MÜLLER, T., NEL, L.H., SCHNEIDER, C., TORDO, N. & WANDELER, A.I. 2008. Can rabies be eradicated? *Developments in Biologicals (Basel)* 131: 95-121.

SCHMIDT, K. & KOWALCYK, R. 2006. Using scent-Marking stations to collect hair samples to monitor Eurasian Lynx populations. *Wildlife Society Bulletin* 34: 462-466.

SETZER, H.W. 1968. Directions for preserving mammals for museum study. *Smithsonian Institution Information Leaflet* 380: 1-19.

SHIER, C.J. & BOYCE, M.S. 2009. Mink prey diversity correlates with mink-muskrat dynamics. *Journal of Mammalogy* 90: 897-905.

SIMMONS, N.B. & VOSS, R.S. 2009. Collection, preparation, and fixation of specimens and tissues. *In*: KUNZ, T.H. & PARSONS, S. (Eds). *Ecological and behavioral methods for the study of bats*. The Johns Hopkins University Press, Baltimore: 849-867.

SIMPSON, G.G. 1931. A new classification of mammals. *Bulletin of the American Museum of Natural History* 59: 259-293.

SMITH, G.C., KAUFMAN, D.W., JONES, R.M., GENTRY, J.B. & SMITH, M.H. 1971. The relative effectiveness of two types of snap traps. *Acta Theriologica* 16: 284-288.

SMITH, M.H., GARDNER, R.H., GENTRY, J.B., KAUFMAN, D.W. & O'FARRELL, M.H. 1975. Density estimations of small mammals populations. *In*: GOLLEY, F.B.,

PETRUSEWICZ, K. & RYSZKOWSKI, L. (Eds). *Small mammals: their productivity and population dynamics*. Cambridge University Press, Cambridge: 25-53.

STUART, C. & STUART, T. 1994. *A field guide to the tracks and signs of Southern and East African wildlife*. Southern Book Publishers, Cape Town: 310 pp.

STODDART, D.M. 1979. *Ecology of small mammals*. John Wiley & Sons, New York: 386 pp.

TWIGG, G.I. 1975. Marking mammals. *Mammal Review* 5: 110-116.

TWIGG, G.I. 1978. Marking mammals by tissue removal. *In*: STONEHOUSE, B. (Ed.). *Animal Marking: recognition marking of animals in research*. Macmillan Press, London: 109-118.

WATT, E.M. & FENTON, M.B. 1995. DNA fingerprinting provides evidence of discriminate suckling and non-random mating in little brown bats Myotis lucifugus. *Molecular Ecology* 4: 261-264.

WEMMER, C., LUNDIE-JENKIN, G., KUNZ, T.H. & MCSHEA, W.J 1996. Mammalian signs (Chapter 9). *In*: WILSON, D.E., COLE, F.R., NICHOLS, J.D., RUDRAN, R. & FOSTER, M.S. (Eds). *Measuring and monitoring biological diversity. Standard methods for mammals*. Smithsonian Institution Press, Washington and London: 157-176.

WILSON, D.E, COLE, F.R, NICHOLS, J.D., RUDRAN, R. & FOSTER, M.S. 1996. *Measuring and monitoring biological diversity. Standard methods for mammals*. Smithsonian Institution Press, Washington: 409 pp.

WILSON, D.E. & REEDER, D.A.M. 2005. *Mammal Species of the World*. 3rd Edition. volumes I+II Johns Hopkins University Press, Baltimore:. 2142 pp. URL: http://www.bucknell.edu/msw3/

WILSON, E.O. 1992. *The diversity of life*. Harvard University Press, Cambridge, Massachusetts: 424 pp.

WORTHINGTON-WILMER, J. & BARRATT, E. 1996. A non-lethal method of tissue sampling for genetic studies of chiropterans. *Bat Research News* 37: 1-3.

YATES, T.L., JONES, C. & COOK, J.A. 1996. Preservation of voucher specimens (Appendix 3). *In*: WILSON, D.E., COLE, F.R., NICHOLS, J.D., RUDRAN, R. & FOSTER, M.S. (Eds). *Measuring and monitoring biological diversity. Standard methods for mammals*. Smithsonian Institution Press, Washington and London: 265-273.

ZIELINSKI, W.J. 1995. Track Plates. *In*: ZIELINSKI, W.J. & KUCERA, T.E. (Eds). *American marten, fisher, lynx, and wolverine: survey methods for their detection*. Gen. Tech. Rep. PSW-GTR-157. Albany, CA: Pacific Southwest Research Station, Forest Service, U.S. Department of Agriculture: 67-89.

7. Appendices

Appendix 1. List of recording elements of capture for small terrestrial mammals.

Abbreviations: ID = identification

Recording capture data	(Micro-) Habitat description
• Field number (consecutive) • Individual ID (in case of recapture) • Date • Collector(s) • Site ID • Check time / Control • Trapline ID • Trap ID • Trap type • Bait used • GPS • Species (Field ID) • Sex: Male, Female, unknown • Age: Adult, Subadult, Juvenile • Reproductive Status: Male: Testes descended or non descended Female: Pregnant, Lactating, Vagina perforated or non-perforated or plugged • Body mass (g), BM • Head-body length (mm), HBL • Tail length (mm), TL • Hind foot length (mm), HFL • Ear length (mm), EL • Tissue sample • Parasite sample • Marking • Remarks: *e.g.* fate (released/recapture/ voucher/dead/kept/marked/ etc.)	**Description of trap location/station** • Trap height • Canopy density • Nearest tree/dbh • Nearest log/stump & diameter • Distance to water • Groundcover (percent herbs, grass, soil, leaf litter, wood, rocks, debris) • Nearest termite mound (in tropical environments) **Inventory of environmental features** • Elevation • Rainfall (last 12/24 hours if rain gauge has been installed) • Temperature (min/max if recording thermometer has been installed) • Humidity (min/max if recording hygrometer has been installed) • Vegetation: ground cover, plant height, plant diversity, stage of maturity, canopy density • Habitat structures (rocks, burrows, soil, logs, termite mounds etc) • Abundance of epigaeic arthropods

Appendix 2. Template data sheet for recording capture data for bats. Abbreviations: BM = Body mass, FA = Forearm length; HBL = Head-body length; TL = Tail length; HFL = Hind foot length; EL = Ear length

Date:																
GPS: Location Data: Habitat: Season:								Moon Phase: Cloud Cover: Types and Lengths of Nets/Traps: Net Opening Hours/ Trapping Hours:								
No.	Date	Species	Site	Time	# Net	# Shelf	BM (g)	Sex	Age	Repr. Status	FA (mm)	HBL (mm)	TL (mm)	HFL (mm)	EL (mm)	Remarks

Appendix 3. List of field equipment for an inventory of small terrestrial mammals and bats.

This list is not exhaustive.

Small Mammals	Bats
Trapping • Traps: Sherman, Tomahawk, (Snap traps?) • Bait: Peanut butter (unsalted), oats • Insulation material: *e.g.* 2 litre milk cartons (tetrapaks) • Bedding material for traps • Pitfall traps (buckets 5 litre) • Funnel (custom-made) • Drift fence (*e.g.* roll of green nylon cord) • Staple gun and staples • Poles • Tape measure (30 m) • Marker tape (biodegradable, non-polluting/brightly coloured / reflective) • Marking pen (water resistant) • Aluminium tags	**Netting** • Mist nets (different sizes) • 3 meter poles for standard ground nets (*e.g.* sectional aluminium or PVC, if they can not be cut in the field) • Stakes • Roles of string • Canopy net unit, freestanding (sectional aluminium poles, ropes, pulley carabiners, large stakes) or: Canopy unit hanging • Sling shot, bow and arrow, crossbow or a line-shooting gun to attach hanging canopy unit • Harp trap kit (additional fishing lines) • Marker tape (biodegradable, non-polluting/brightly coloured/ reflective)
Treatment of animals • Gloves (firm to bites) • Disposable gloves • Plastic bags (3 litre size) or cloth • Measurement tools (ruler, calliper) • Spring balances (10 g, 30 g, 100 g and 300 g); or larger ones (1 kg, 5 kg) for animals caught in Tomahawk traps • Tubes 5-6 (15-50 mm diameter, length 15-25 cm) from acrylic glass • Cotton wool • Marking tools (if requested) • Cage • Field book • Identification keys	**Treatment of animals** • Gloves (firm to bites) • Linen or cotton capture bags • Measurement tools (ruler, calliper) • Spring balances (10 g, 30 g, 100 g and 300 g); or larger ones (1 kg) for Megachiroptera • Field book • Identification keys
Tissue taking in the field • Scissors, forceps • DNA tools: vials … • 95% Ethanol for DNA tissue (or DMSO)	**Tissue taking in the field** • Forceps • Syringes and needles • Heparinised hematocrit tubes • Microscope slides • Biopsy punches & 2 ml vials • Filter paper • Lighter (for forceps sterilization) • Methanol (fixation of blood smears) • 95% Ethanol for DNA tissue (or DMSO)

Specimen preservation dry/wet

- Container/jar (large, tightly sealing) for killing
- Shallow plastic containers (“Tupperware”)
- Wide-mouth barrel (CurTec wide neck kegs)
- Disposable gloves
- Plastic bags (3 litre size) or cloth
- Inhalation anaesthetic (*e.g.* Isofluran)
- Formalin
- 95% (75%) Ethanol for whole body preservation
- Cintillation vials (leak-proof 5 ml)
- Scissors, scalpel
- Labels (fluid-submerged) of 100% rag paper
- Tags
- Thread or twine for tags
- Permanent ink (*e.g.* Pelican fine drawing ink or similar)
- Board or styrofoam sheets
- Pins, wire rings, needle
- Wires of differing thickness, wire cutters
- Cotton wool, quilting cotton, long fibre cotton
- Maize meal
- Desiccants (Silica Gel-type)
- Screen cage

Other

- Hand-held GPS unit
- Digital camera/ ideally SLR with Macro lens & flash
- Binocular
- Headlamps, additional flash lights
- Spare batteries of all needed sizes (D cell, AA, AAA etc.)
- Buckets, bowls, strainer, measuring pitcher and funnel
- Equipment case (waterproof, firm, well-arranged) *e.g.* light toolbox
- Rucksacks/bags for transport of material (*e.g.* traps)
- Tools: spade, pliers, shovel, hammer or small hatchet for stakes, hoe or pickaxe in tough soils
- Disinfectant

Optional

- Bat detector (Anabat, Pettersson etc.) & cassette recorder or CF cards
- Folding table/ Folding chairs
- Warm clothes (it can get quite cold at night; even in tropical areas)
- Bug repellent

Habitat assessment

- Altimeter (not necessary if GPS available)
- Spherical densiometer
- Fiber glass diameter tape measure (width 16 mm, length 10 m)
- Folding rule

Chapter 20

Sampling Amphibians and Reptiles

by

Xavier Eekhout
Department of Biodiversity and Evolutionary Biology
Museo Nacional de Ciencias Naturales (CSIC)
José Gutiérrez Abascal 2, 28006 Madrid, Spain
Email: xeekhout@mncn.csic.es

Abstract

Amphibians and reptiles rank among the most charismatic vertebrates and have received a lot of taxonomic attention over time. Nevertheless, to improve the speed and quality of inventory and monitoring of these animals, we here provide an overview of the different sampling techniques used per taxon and detail the different ways of handling amphibians and reptiles. Also, we indicate the different types of data that are to be collected from specimens. Throughout the chapter tips and trick for safety are also provided.

Key words: amphibians, reptiles, sampling methods, handling methods, safety

1. Introduction

Amphibians and reptiles are important components of ecosystems. Recently, there is an increasing awareness that, like for many other taxa, herpetological diversity is threatened, and the growing list of declining populations suggests a worldwide crisis (Blaustein *et al.*, 1994). Efforts to collect baseline data about occurrence, distribution, and status of populations are relatively well advanced for amphibians, but much less so for reptiles for which recent data suggest that turtles and crocodilians are as threatened as anurans (Gibbons *et al.,* 2000).

In this chapter, we review field techniques for sampling reptiles and amphibians. However, as good body of literature that addresses in detail different topics of the sampling of reptiles and amphibians (see Heyer *et al*., 1994; Dodd, 2009; Graeter *et al*., 2008; Kok & Kalamandeen, 2008 and the references there contained) already exists, this work is only a general manual useful in ATBI initiatives. As such, it does not go into detail of all the aspects that need to be considered when inventorying and monitoring reptiles and amphibians.

Inventories may range from quick short-term surveys to extensive long-term monitoring programs. Inventories ideally involve the use of many collecting and observational techniques, in addition to the compilation of data from past surveys obtained from the literature and museum specimens. Monitoring programs ideally should use standardised techniques so that statistical analysis of data becomes possible.

2. Inventory and monitoring techniques for amphibians and reptiles

This is a condensed review of the most common techniques used in inventories and monitoring of amphibians and reptiles whereby we focus on the possibilities, limitations and materials needed.

2.1. Standardising the sampling effort

In monitoring, the importance of having standardised sampling procedures cannot be overemphasised. The methods mentioned hereunder are suitable for both terrestrial and aquatic habitats, and may be an efficient way of standardising monitoring surveys (*e.g.,* Brown 2001; Meik *et al.,* 2002). Next to recording biological data, the environmental conditions must also be scored and this again in a standardised way.

Standardisation of sampling can be achieved through: (i) time-constrained searches; (ii) area-constrained searches; (iii) quadrat sampling; (iv) transect surveys.

2.1.1. Time-constrained searches

The premise behind this technique is to actively search for animals in a given area for a pre-defined amount of time. If additional information will be collected from the animals found (*e.g.*, body measurements or marking individuals), then the time invested in these activities should not be considered as part of the search. Time-constrained searches are mostly applied during terrestrial surveys, although they can also be used in aquatic habitats, particularly for amphibians.

The main limitation of a time-constrained search is the long periods that the survey participants must commit to it. Furthermore, it must consider that the results of time-constrained searches are highly influenced by environmental factors such as time of the day, season, and weather (*e.g.,* it is well known that amphibian activity increases very much after rainfall). Another factor that will heavily influence time-constrained searches is the level of experience of the surveyors. Experts are likely to find more animals than inexperienced workers. It is vital to keep these factors in mind when designing a study.

For an inventory it is advisable to repeat the sampling to include several days with different weather conditions and to always follow the same previously planned search routine (*e.g.,* if the first search included turning stones, then that should also be included when repeating searches).

2.1.2. Area-constrained searches

With area-constrained searches the search is focused on a certain area and not on an amount of time. Area-constrained searches will give information in terms of absence or presence of species, and potentially some data on life history of the species such as time of reproduction, activity patterns, and habitat use. The size of the area to be searched might vary but it will depend either on the habitat type (*e.g.*, pond, creek, meadow, etc.) or on the focal species.

The main limitation of this technique rests with the effect of environmental conditions, the experience of the workers, and the planned search routine. As with time-constrained searches, the searches should be done during several days with different weather conditions or even different seasons to maximize the chance of encountering all species present in the area.

2.1.3. Quadrat sampling

In this technique, sampling arrays in a study area must be randomly distributed and the absence or presence of animals in these arrays verified. The sampling areas are usually squares (quadrats) that are thoroughly searched (Jaeger & Inger, 1994).

The main drawback of quadrat sampling is that the setup can be very time-consuming.

Within quadrat sampling, we can differentiate point sampling where small squares are used, and broad sampling where larger quadrats are used (Kok & Kalamandeen, 2008). Point sampling is preferred when studying single species

in which the individuals are relatively small and densely distributed, while broad sampling is applied to species that are widely dispersed, large bodied or both, as well as for multispecies assemblages. In both cases, all quadrats need to be of the same size within each study area. A modification of this technique is called 'patch sampling', in which the sampling arrays are normally specific microhabitats (*e.g.,* logs, bushes, etc.). Patch sampling is applied when looking for specific target species, which we know or suspect that are confined to specific microhabitats within a larger habitat (Jaeger, 1994a). For both techniques some pre-requisites have to be met.

For quadrat sampling:

- Animals may not leave the quadrat before being observed.
- The quadrats are randomly distributed.

For patch sampling:

- Each patch must be defined precisely and in an operational way.
- All patches must be equally locatable by the observer without any bias.
- Animals may not leave the patch before being observed.

If these criteria are met, then quadrats and patches can be distributed randomly within the study area. Each of them then represents an independent sample, allowing statistical analysis of the obtained data if at least 25 to 30 quadrats were scored (Jaeger & Inger, 1994).

Quadrat sampling has proved to be particularly useful in forests when searching for ground-dwelling amphibians and reptiles (Rodda & Dean-Braley, 2002). For best results in this methodology of quadrat (or patch) sampling, it will be important to apply the most appropriate searching technique within each of the quadrats (*e.g.*, using a rakes over leaf litter).

2.1.4. Transect surveys

A linear transect is established and the whole narrow strip (and nearby areas) is searched for animals. This is usually utilised for surveying herpetofauna across environmental gradients but can also be used within a single habitat (Jaeger, 1994a). However, for homogeneous study areas, quadrat sampling is recommended. If the design is properly randomized this method will provide a good representation of the occurring fauna over all habitat types. Depending on how the transects are set regarding the gradient, different information will be obtained. If transects are set in parallel to the gradient studied, then these surveys may be used to compare species across habitats. If on the other hands, transects are set perpendicularly to a gradient (*e.g.*, along a river), then one will be able to study changes in parameters of a given species along the gradient. The most common scale used in transect surveys is at the habitat level, but it is possible to work on a larger scale (ecosystem or landscape) by using, for example, aerial surveys across a large transect (Mourão *et al*., 2000).

Ideally, transect surveys have to meet the following assumptions:

- Specimens are randomly distributed throughout the transects.

- Transect lines are randomly chosen.
- All the specimens in the transect will be observed.
- Animals will not be counted twice within a transect and among transects.

When preparing transect surveys it is important to consider that some species will not meet all the method's assumptions. For example, cryptic species will not be observed or will flee from the observer without any notice, or many species do not have a random distribution, as they are associated to specific microhabitats.

2.2. Sampling Techniques

For selecting the most suitable sampling technique, it is necessary to evaluate :

- The objective of the study.
- The conspicuousness of the species of interest (their activity and habitat).
- The cost, time, and resources needed.

By and large, the methods that are more time- and resource-intensive will yield most information, and will allow more powerful statistical analyses. However, depending on the goal of the study, such intensive methods might provide data that are not needed (*e.g.,* obtain detailed ecological data when presence or absence of species would suffice). Furthermore, it is also more productive to use a combination of techniques instead of applying a single one, but again this will require more resources. Therefore, one must strike a balance between available resources for research and desired results before starting fieldwork.

The most common techniques used for sampling reptiles and amphibians can be divided into active and passive sampling, each with a number of specific techniques.

2.3. Active sampling

2.3.1. Visual encounter surveys (VES)

VES is by definition a time-constrained method in which observers sample for species richness and abundance along a survey path (Crump & Scott, 1994). The time spent in the field and the numbers of observers are taken into account. This technique is appropriate for both inventory and monitoring. VES might be particularly useful for detecting rare species that seldom fall into traps, and thus by using VES in combination with a passive sampling technique, it is potentially possible to obtain the complete species composition of the sampled area. Nevertheless, the efficiency of VES will vary much depending on the type of habitat (*e.g*. low vs. high vegetation) and the species biology (*e.g.* fossorial vs. arboreal). As a matter of fact, visual encounter surveys have a number of assumptions that in many occasions cannot be fully met :

- Each individual of every species must have the same probability of being encountered. This will not be met for example in species with a large sexual dimorphism were one of the sexes is much more visible than the other;

- Each individual is only recorded once during the survey. For this the use of individual marking may be the solution, but it implies a higher time investment;
- Each observer doing the survey must have similar experience and be able to potentially obtain the same results. The best approach to this problem is by training the workers in advance to ensure a similar level of experience.

Road cruising and aerial surveys could be cited as visual encounter surveys, although these are done at a different scale and have specific characteristics. In the case of road cruising, a road is used as a survey transect that is methodically driven through looking for both alive and roadkill specimens (Andrews, 2008). Aerial surveys are mostly used for estimating population size and distributions of large-bodied reptiles such as crocodilians or sea turtles (Glaudas, 2008).

2.3.2. Dipnetting and kick sampling

We refer to dipnetting when a dipnet is swept through an aquatic habitat to capture herpetofauna. When the dipnetting process is semi-standardised – the number of sweeps is recorded and compared among habitats – one may call it sweep sampling (Dodd, 2003).

Sweep sampling is used for sampling herpetofauna in small aquatic habitats (treeholes, springs, puddles, and ponds) where it is more efficient. However, sweep sampling may be used in larger aquatic habitats such as lakes as well with the aid of seines and nets. The main targets of this technique are amphibian larvae. It is important to consider that specific differences in animal positioning in the water column may result in differences in the ability to catch different species. Also not all species can be caught with the same net, so the type of net and its mesh size must be carefully selected depending on the ecology and size of the targeted species. Moreover, dipnetting should be scheduled in the season when the species are most likely to be found in the water. When these factors are taken into consideration sweep sampling may be a very effective sampling method that allows for comparisons among aquatic habitats that are somewhat homogeneous.

Kick sampling is a technique that is especially fit for aquatic habitats of small to intermediate size and with fast flowing current. It is predominantly used when looking for stream dwelling amphibians. It consists of lifting and removing all loose substrate from a stream bottom, kicking loose pebbles or even hand raking everything into a net. Typically, the most common nets used are those with a D-frame whose flat side may rest on the bottom of the stream. The most efficient way to kick-sample is with two-person teams, where one worker loosens the woody debris, rocks and other substrate, while the other holds the net in place. It is very important when sampling for herpetofauna to check the nets very often (every 5 minutes or less) to decrease animal stress and mortality. As before, mesh size has to be considered based on the target species size. The smaller the mesh size, the more species will be captured, but small mesh nets tend to clog up faster with debris, and thus require more frequent maintenance to maintain efficiency.

The main limitations of kick sampling are that it is very labour intensive and that it can cause habitat disruption. For the latter reason, it is very important to redeposit the habitat items (*e.g.* large stones, wood debris, etc.) that had been moved.

2.3.3. Stovepipe sampling

Stovepipe sampling is a quantitative method in which aquatic animals are trapped within an enclosure and later removed from it with a net (Shaffer *et al.*, 1994). The enclosures or samplers are typically pipe-like (one may use air conditioning ducts, culverts, stove pipes, and PVC pipes) (*e.g.,* Alford, 1986 or Skelly, 1996) or a rectangular box (*e.g*., Harris *et al.*, 1988). These samplers are placed in the water, firmly set against the substrate, but with enough care as to not disturb the environment and cause the animals to flee. Once the sampler is in place, a net is swept within the enclosure to collect the animals. This technique is especially suitable for obtaining quantitative estimates of larval densities that can be used to estimate population size. Samplers should be placed randomly across the habitat, and their dimensions and the water depth recorded to obtain values of captured animals per volume. The best habitats to apply stovepipe sampling are shallow waters with sandy or mucky substrates, which allow to easily install the samplers. In habitats with water vegetation, pipe enclosures are easier to install than rectangular ones. This technique can be time intensive to use, so in case of large habitats or if we only want to determine the presence of a particular species, other methods such as dipnetting will be more useful.

2.3.4. Egg mass and nest counts

This is a method that can be used during breeding periods to monitor the reproductive activity in reptile and amphibian populations. In amphibians, egg masses are counted around a pond perimeter or within the pond and it is particularly useful for explosive breeders and those that reproduce in communal aggregations. For identification purposes it is recommended to photograph the egg masses or at least use detailed language to describe it. Mitchell (2000) recommends making the following observations:

- Is the mass globular or round?
- Are the eggs clumped, separated or on a string?
- What colour and shape are the embryos?
- Is jelly surrounding the eggs firm or loose?
- Is there a film on the surface of the mass?
- To what type of vegetation is the mass attached?

In the case of reptile egg nest counts, this technique is most useful for turtles and crocodilians. Normally a relatively large area must be checked and there is need of having some previous knowledge of nesting grounds, and sometimes the recognition of tracks can be very useful, as well as the leftover from predation over the nests or the remnants materials after the babies hatch (*e.g*., broken egg shells). Egg mass and nests counts is a relatively simple and powerful method for determining the presence of species, and especially in the case of species

that lay a single clutch per year it can be a reliable indicator of population size. This technique is nevertheless useless for amphibians that lay eggs in the land and for most squamata reptiles. Finally, it is important to consider that the lack of egg masses or nests cannot rule out the possibility of a species being present, but not reproducing.

2.3.5. Auditory surveys

Auditory surveys are very useful for estimating species richness of anurans. Male anurans in particular tend to be fairly conspicuous during breeding season when the use their mating calls for attracting females. These calls are species specific, so during the breeding season listening stations can be randomly selected along the breeding site to identify species presence and their relative abundance. This technique has the advantage of easily covering rather large areas while being hardly non-invasive.

Not all anurans are equally easy to detect, but with some training even non-expert workers can obtain good results. In inventory, regular auditory surveys are very helpful for determining species composition, but there are some limits when it comes to monitoring changes in a population because there is always a bias towards only observing declines in calling activity and it is difficult to evaluate if these are due to natural fluctuations. If the aim is monitoring, acoustical surveys should always be coupled with other sampling techniques. In the chapter on bioacoustics more information can be found.

Fig.1. Calling frog. (Photo by author).

2.3.6. Basking surveys and basking traps

Sampling techniques based on the animal's basking activity are applied in aquatic habitats, especially rivers were the observer can advance in parallel to the river bank while scanning basking sites with binoculars. The studied animals are normally turtles (Buhlmann & Vaughan, 1991; Lindeman, 1998), although it has also been applied on water snakes (Mills *et al*., 1995).

Apart from species presence, basking surveys can also give information on sex ratios and juvenile recruitment, but when further information is needed, this technique is to be complemented with basking traps. These are wire traps that

are attached to the underside of the basking log so when the animal instinctively jumps into the water, dives to the bottom of the trap giving the observer time to retrieve it. It is important to remember that basking traps must allow the animal to ultimately climb out of the trap if they fall in and the researcher is not present. The effectiveness of the basking surveys will depend on the amount of basking surface available, the time of the day or season when it is done and the animal's basking behaviour. The main limitation of this technique is that it depends on amount of basking surface available. If there are no basking sites, then no animals are observed, but it does not mean that the species is absent. For using basking traps it is absolutely necessary to identify first favourite basking sites, so a basking survey will always precede the setup of basking traps. In monitoring initiatives basking surveys and basking traps should always be made in conjunction with mark-recapture studies.

2.4. Passive sampling

2.4.1. Artificial cover

Many reptiles and amphibians use covers in the wild for hiding. Logs, rocks and even human debris provide refuge to many species, which implies that sampling these covers many times is an effective method. The problem with these "natural" covers is that quantifying their effectiveness is difficult.

By using artificial coverboards we can standardize the sampling effort maintaining the natural habitat and limit biases. The materials most commonly used for coverboards are solid wood boards, plywood boards, corrugated metal strips, tarpaper and horticultural plastic sheeting. These coverboards are set in array designs as linear transects, rectangular grids or webs, depending on the species and/or habitat sampled. Artificial coverboards have been used to sample many species of reptiles and amphibians (Parmelee & Fitch, 1995; Sutton *et al*., 1999; Houze & Chandler, 2002; Ryan *et al*., 2002; Smith *et al*., 2006). An additional benefit when using coverboards is that as they do not restrict movement, it does not require continuous surveillance as for example pitfall traps. Their maintenance is also easy and inexpensive when compared to pitfall traps. In studies where coverboards and pitfall trap arrays have been used, pitfall always captured more species and more individuals (Sutton *et al.*, 1999; Ryan *et al*., 2002), although coverboards detect species that are not found in pitfalls. In this sense this technique has proved to be particularly useful for small secretive snake species (Fitch, 1992).

When checking coverboards it is advisable to use tool such as snake hooks to avoid accidental bites. It is also advisable to flip the coverboards always towards the researcher to avoid the animals to escape. Finally, when sampling the coverboards it is also advisable to record environmental data such as the weather conditions, time of the day or the temperature. Sampling encompassing as many environmental conditions as possible will always yield better results.

2.4.2. Polyvinyl chloride (PVC) pipe surveys

PVC pipes are an easy and inexpensive technique for sampling hylid tree frogs. These PVC pipes can be placed in the ground or mounted on trees following a grid or transect setup. The ground-placed PVC pipes can be used nearby the breeding areas of the hylids or as a complement to pitfall traps and drift fences, which are normally easy to avoid for the tree frogs. The tree-mounted PVC pipes on the other hand are suitable for sampling the tree frogs even outside their breeding season (Dodd, 2003). For ground-placed pipes a good length is 1 m vertical pipe with around 60 cm sticking out of the surface, while tree-mounted pipes can be of around 60 cm with the bottom part set at a height of 2-4 m. These pipes should have the bottom sealed with a cap to retain some water, but holes should be made in the pipe at about 15 cm to allow draining the excess of water. A good average diameter for the pipes in both cases is about 2-5 cm. Nevertheless, tree frogs can be of many different sizes, so it might be necessary to try out pipes with different sizes and diameters until finding the most successful design for a given species. An important benefit of PVC pipes is that it causes no mortality on the sampled animals, so the frequency and timing of the checks can be very flexible. This allows accommodating this technique easily with other activities and also makes it suitable for using in remote field sites. The main limitation it has is that it is very specific (only for tree frogs) and that PVC pipes are rather conspicuous, so they can be subject of theft or unwanted manipulation. This technique is most useful for detecting presence/absence of species, and even for determining timing and dispersal from breeding grounds. On the other hand it is very tricky for comparing between sites because its results will depend very much on species assemblage and on the availability of other natural hiding sites. If the aim is monitoring through time in a same site, PVC pipes in conjunction with marking individuals can give much information.

2.4.3. Leaf-litterbag surveys

Leaf-litterbag surveys are specific for salamanders, which can be difficult to monitor due to their cryptic and fossorial nature. Litterbags have been commonly used for many years to estimate leaf litter breakdown in streams (*e.g.*, Peterson & Cummins, 1974), but it has been adapted for sampling stream-dwelling salamanders (Pauley & Little, 1998). This technique was successfully applied in the Great Smokey Mountains National Park to inventory various streams (Waldron *et al*., 2003). Their basic design consists of a square (50-90 cm per side, with 70 cm x 70 cm being the optimal size) piece of plastic netting with 1.9 cm mesh. Small rocks are placed on the netting in the field and covered with leaves before the corners are brought together and bound with cable ties to form the litterbag. Finished bags are placed in the stream at regular intervals and after an acclimation period of a couple of weeks, each bag is checked by placing a dip net underneath and lifting the bag into a bucket of water. Then, to extract the salamanders from the bag, dip the bag repeatedly in the bucket and then pour the water through the dip net. The salamanders are then processed and the bags are placed back into the stream.

Although this technique has proven to be successful for detecting the presence of salamander species, it is not capable of indexing populations sizes, so it cannot be applied on its own in monitoring programs.

2.4.4. Aquatic and terrestrial funnel trapping

Funnel trapping is a standard method for trapping many groups of animals including reptiles and amphibians. The principle behind these traps is pretty simple: animals are directed trhough a small opening in the trap via a funnel or ramp, and once inside, are unable to find their way out. This is a technique especially useful for capturing rare cryptic species and has the advantage of being suitable for standardizing. In addition as traps are used during a lapse of time, this technique is also less sensitive to biases resulting from temporal variations. On the other hand funnel trapping requires a substantial investment of time and equipment. The traps themselves can be expensive, and should be checked often to avoid mortality of the trapped animals. When applying funnel traps in inventories, the effort should focus on habitats and times when the target animals are more likely to be active, and the more different habitats sampled the more species we will likely detect. Nevertheless, funnel traps have generally a low capture rate, so for successful inventories, a high intensity sampling is recommended (several hundred trap-nights spread across the season).

Funnel traps are also very useful in long term monitoring programs as the trapping scheme can be easily replicated allowing comparisons. For this, traps can either be set in systematic or random arrays. Based on the capture rates detected we will be able to infer population status, but always with some reserves, as capture rates will depend not only in population size, but also in level of activity and the propensity of the species to enter and remain in the traps. The ideal situation is when traps are complemented by mark-recapture data.

Funnel traps can be used in aquatic and terrestrial habitats, and can be of different sizes, materials and shapes. The use of one or other will normally depend on the target species:

- **Small aquatic funnel traps**: These can be either cylindrical or rectangular and are normally used for trapping water snakes and aquatic amphibians. The traps that are commercially available are designed for capturing crawfish or eels, but these can also be used for amphibians. They are typically double ended and built of steel hardware cloth, plastic or nylon mesh. The plastic traps are normally the most suitable for trapping the smaller species. As an alternative, small and inexpensive traps can be made by inverting the top of a plastic soda bottle and anchoring it to the substrate with a stake (Willson & Dorcas, 2003).
- **Hoop-nets**: These are large funnel traps used primarily for trapping highly aquatic carnivorous turtles, although it is potentially useful for trapping any aquatic turtle. These traps are also commercially available in different sizes and made of twine or mesh. In their setting, the traps normally have a part above the surface allowing the captured turtles access to air. Normally hoop nets are baited to increase success and should be checked at least daily. In

occasions hoop nets can also capture large aquatic salamanders and large snakes.

- **Interruption traps and fake nets**: These traps are suitable for complementing the hoop nets. In this case the trap is unbaited, but uses nets or natural channels to draw the turtle towards the funnel. Essentially they work like drift fences but on the water. The design of the trap can include unbaited hoop-nets, swing door traps or pressure plate traps at the end of the channels of nets. As with the hoop-nets, although these traps are mainly for turtles, they can capture other species such as large amphibians or large snakes (Vogt, 1980).
- **Terrestrial funnel trapping**: Terrestrial funnel traps are typically used in conjunction with pitfall traps along drift fences. The design of the trap can be very variable, although the most common variation consists of a wire hardware cloth cylinder with inverted hardware cloth funnels pinned into each side (Fitch, 1987). It is advisable to set the traps in the shade or cover them with a board to make them more attractive and to protect the captured animals from the rain and the heat. In the case of amphibians it is also advisable to use some kind method to moisture the inside of the trap (*e.g.*, a moist sponge). Terrestrial funnel traps can also be constructed of wood boxes, which makes their building more complex and time-consuming, but in different studies have proved to capture almost any snake, reptile or amphibian possible (*e.g.*, Burgdorf *et al*., 2005; Enge, 2001; Greenberg *et al*., 1994).

2.4.5. Terrestrial drift fences and pitfall traps

Drift fences have proven to be effective for sampling most amphibians and squamata reptiles (Nelson & Gibbons, 1972; Semlitsch *et al*., 1981; Hanlin *et al*., 2000; Enge, 2001; Russell *et al*., 2002; Ryan *et al*., 2002, Todd *et al*., 2008). The basic design of a drift fence is a straight fence buried slightly below ground, and standing up to 50 cm high. Pitfall traps are then buried at floor level and placed at a certain interval alongside the fence. The spatial arrangement of the fence can vary, and we can separate drift fence arrays into:

- **Straight-line drift fences**: These can be set up in X or Y-shaped arrays and are normally used for sampling upland habitat (Corn, 1994).
- **Continuous or partial drift fences**: This setting is commonly used to circle partially or completely wetlands (Dodd & Scott, 1994).

The capture rates and effectiveness of this technique may differ very much between sites, but it is clear that this technique is particularily useful for determining species richness and relative abundance (see Ryan *et al*., 2002 for comparisons with coverboards and time-constrained visual surveys). The main limitations are as follows:

- Expensive and hard to set up. After installing, the traps should be visited at least once in a day.
- Capture biases. Some species may show trap avoidance or even attraction towards the pitfall traps.

- Many species such as large snakes or tree frogs can escape from the pitfall traps. This can be somewhat avoided with putting plastic collars on top of the pitfall traps or using double-pit systems.
- Species associated to certain microhabitats might not be sampled.

The best way to improve the success of drift fence arrays is to combine pitfall traps with funnel traps. This technique is normally used on long-term monitoring programmes due to the relatively high amount of time and funding needed to install them.

3. Capturing and handling animals

When sampling animals they should be handled in a way that allows further study (vouchering, photographing, marking, etc.). Handling is generally done by hand, but several tools and utensils can ease the task and increase the safety of both the sampler and the specimen.

3.1. Snakes

Prior to identification, all snakes should be considered potentially venomous. When identified as venomous, ONLY EXPERIENCED AND TRAINED PROFESSIONALS SHOULD EVER ATTEMPT TO CAPTURE AND HANDLE THEM.

The most common tools used for capturing snakes are hooks and tongs that are used to immobilize the snake and keep it at a safe distance from the researcher. The usual procedure for manipulating a snake is using the hook or tong for lifting up the animal gently from the mid-front body while keeping hold of the snake's tail to avoid it from turning around.

For hand-catching snakes, we should set it in an open area and press its head gently, but firmly against the floor, using for example the bottom of the hook, so we can safely manipulate it. We can secure the head between the thumb and fingers of one hand, and use the other hand to sustain the rest of the body weight to make sure the snake does not suffer spinal injuries. Although giant snakes (boas and pythons) are not poisonous we should never underrate their strength. They should never be handled by only one person and special care must always be paid to their heads. These animals need to bite in order to strangle and their bites can easily infect due to the bacteria in their mouth.

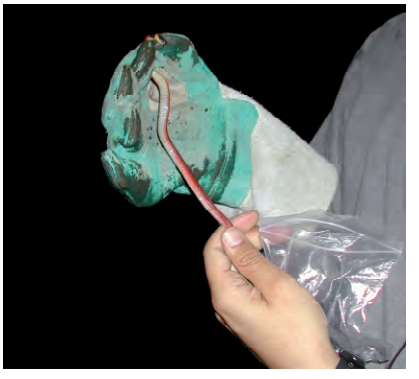


Fig. 2. Handling a small snake. (Photo by author).

For smaller harmless and fast moving colubrids, hooks and tongs might not be appropriate and collecting directly by hand with thick protection gloves is recommended.

Hooks can easily be handcrafted, but tongs are more difficult to manufacture and are normally purchased from supply companies. Currently both hooks and tongs from different brands are readily available through the Internet. They should be made of a light but resistant materials such as anodized aluminium or titanium. The size of the hook and tongs will depend on the size of snake we target. The handles of both tongs and hooks should be made of a material that will not slip during the manipulations, such as rubber. Finally the material that will be in contact with the snake should minimize the chance of injuring the snake while manipulating it (*e.g.*, rubber coated).

3.2. Lizards

Lizards on average can be quite difficult to capture by hand due to their size and fast movements, so to assist on their capture we can use a small noose. The noose can be built with a long, slender pole such as a bamboo stick or a telescopic fishing pole where a thread of dental floss or fishing line can be attached. It is common lizard behaviour to flee upon sensing something approaching and then freeze shortly, and it is then when the noose can be placed over the head to trap the animal from a certain distance (see Marcellini & Jenssen, 1991). In the case of large lizards, caution must be taken when handling as they can cause injuries with their claws, and deliver powerful bites that can easily become infected. It is recommended to manipulate these animals wearing heavy-duty gloves to prevent any possible wounds. It is very important to avoid capturing lizards by the tail as it will break off in many occasions.

3.3. Aquatic turtles and tortoises

Aquatic turtles can sometime be captured by hand and with the aid of a dip net, although the usual way of capturing turtles is using traps (see survey methods). In the case of turtles or tortoises they should always be handled with care as they can deliver powerful bites, but this is easily avoided by keeping your hands away from their head. Normally turtles can easily be held at mid- or back-body, although additional attention should also be paid for some species' claws that can be elongated and inflict deep wounds. As with large lizards the use of thick gloves to manipulate the animals is also recommended.

Fig. 3. Handling turtles. (Photo by author).

3.4. Crocodilians

Due to their size and dangerous bites, crocodilians should exclusively be handled by experts. Normally their capture is done by several people and with the aid of a noose. While small and young animals can be grabbed from behind the head with one hand, using the other hand to support their weight (as you would do with a large lizard), larger animals have to be handled by several people. It is important to make sure that the jaws are closed, for example by wrapping duct tape around them, before doing any measuring, and extreme caution must be paid to the tail which can deliver powerful strokes. It is highly advisable to cover the animals' eyes to reduce their stress.

Fig. 4. Handling large animals. A. Small crocodilian; B. Large lizard. (Photos by author).

3.5. Amphibians (frogs, toads, newts, salamanders and caecilians)

Aquatic amphibians can be captured by hand and with the aid of a dip net before they jump into the water or while floating in shallow waters. Most amphibians are nocturnal, so a flashlight can also be used to temporarily blind them and get close enough to them. In the case of terrestrial amphibians the challenge is locating them, as on average capturing them by hand is not difficult. Nevertheless we should have in mind that all amphibians have some degree of toxicity in their skins. Cutaneous glands are a shared character of all adult amphibians and they are normally the main source of biological active compounds found in the amphibians skin. The level of toxicity depends on the exact components of these substances and can range from noxious to highly toxic depending on the animals. The highest toxicity is due to the presence of alkaloids that in most cases derive from the arthropods the animals eat in the wild. Alkaloids have been found in some salamanders, but especially in Dendrobatidae and Mantellidae (Daly, 1998). The secretion of these compounds will be increased when the animals are stressed due to handling so the use of latex gloves or an inside out Ziploc bag is recommended to avoid direct contact with the skin. If none of these are available, and we must necessarily have direct contact with the animal, hands should always be thoroughly washed after manipulating them, making sure we avoid contact with our eyes or mouth. In the same way, any surface that has been in contact with the animals should be thoroughly rinsed and cleaned with water.

Fig. 5. Stressed frog. (Photo by author).

For safely handling frogs and toads, they should be held between the fingers and thumbs around the waist of the animal. For some specific measurements or for photographing the frogs should be grabbed from one of the front legs between the thumb and index finger while sitting on top of the hand. The grab should be firm enough to avoid the animal from escaping using their strong back legs, but with much care to avoid any damage to the front limbs. In the case of salamanders and newts, we should hold them in the entire hand gently restraining the animal between the thumbs and fingers just behind the head, in a similar way as it is done with medium and small-sized lizards.

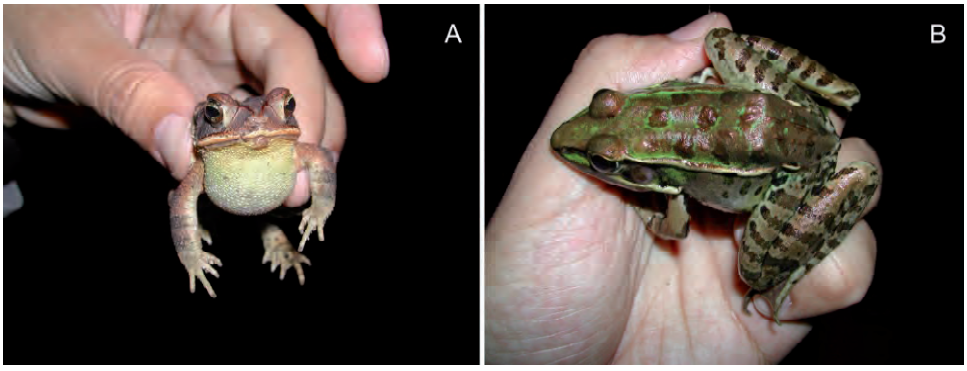


Fig. 6. Handling frogs. A. Holding the animal safely; B. Handling position for measurements. (Photos by author).

Finally, it is important to consider that when handling different amphibian specimens in the field, a researcher can involuntary become a vector for transmitting pathogens such as chytrid fungi. The chytrid fungus *Batracochytridium dendrobatidis* is behind the disappearance of entire populations of amphibians around the world, so if your are going to handle amphibians in the wild, there are a number of rules you should strictly respect to avoid the transmission of chytrid fungi between populations or sites:

- Never move individuals of adult amphibians, larvae or egg between distinct places even if they are very close since this could contribute to the dispersion of the pathogens;
- Never introduce animals, plants or any other organism in the environment, because, besides interfering with native species, they may carry pathogens. We know that fish can transmit viruses that affect amphibians, and in many countries the native amphibians are infected by introduced amphibian species that carry the chytrid fungus. If you detect introduced (allochtonous) organisms in your area, get in contact with an expert;
- Avoid accidentally transporting the pathogens yourself. The chytrid fungus does not have a stage that is resistant to desiccation but it can survive in whatever type of organic material that maintains humidity. As such, after a trip to the field wash well at the site all the objects that have been in touch with the environment (*e.g.*, boots and sample nets). After submerging them in bleach (a bath of 30 seconds is sufficient if you use domestic bleach with at least 4% sodium hypochlorite) or in other suitable disinfectants put them out in the sun for as long as possible;
- If you do not want to use bleach to clean your field material, you can use commercial products specifically sold in veterinary stores. Some suitable commercial products are: Halamid® (www.alpharmaanimalhealth.co.uk) and Virkon® (www.antechh.com);
- If you hold amphibians use disposable gloves or if it is necessary to keep them for some time use disposable containers or ones that have been previously sterilised. Do not put them in touch with specimens from other areas if you are going to return them to the natural environment. Remember that you must sterilise all equipment before using it;
- Inform when possible about the problem of emerging diseases in amphibians and how it is possible to avoid contributing to its spread.

4. Transporting and housing captured animals

If the captured animals must be transported to the lab and housed for some time it is necessary to use appropriate containers.

In the case of amphibians it is most important to keep them in moist substrate in containers or sealed plastic bags. It is a good practice to include some leaves or leafy branches to prevent squashing and maintain humidity. A moist paper towel or standing water in the container usually is effective depending on the needs of the species in question. For tadpoles, plastic containers filled in with water from the capture site can be used, and these containers should be transported in lightly chilled coolers to keep the tadpoles with a relatively low metabolic rate.

Small containers with ventilation are useful for holding small snakes, small turtles, and most lizards. Cloth bags of all sizes, including pillow-cases, are useful for temporarily holding even the largest lizards, turtles, snakes, and small crocodilians. One must be careful not to allow the animals to suffocate or drown while transporting them, and avoid placing them in direct sunlight where any container can rapidly overheat and the animals inside die.

Once in the lab, the setting prepared for short term housing the animals can be very simple. One must make sure that the temperature is suitable for the animals, that natural photoperiods are respected and that the containers are clean and have sufficient water and food.

5. Collecting information from captured animals

5.1. Measurements

All amphibians, squamata reptiles (lizards and snakes) and crocodilians the standardized measure used is the snout-vent length (SVL) that is defined as the distance between the tip of the head and the end of the cloaca. In addition, the tail length can also be recorded to have the total length of the animal, but salamanders and squamata reptiles have the ability to loose their tails as a defensive mechanism upon being attacked by a predator.

Together with the measurements of the body length, the typical measurement is weight. Most herpetofauna can be weighted with either a spring scale or an electronic scale, but for larger species (giant snakes, crocodilians, large turtles) a truck scale will be necessary.

Due to the ectotherm nature of reptiles and amphibians, in many occasions, it will also be of interest to obtain the cloacal temperature of the animals. Ambient temperature can be used as an approximation if it is not possible to measure body temperature, but it must be remembered that there can be significant differences between both measurements due to fluctuations that the animals metabolism can produce in their body temperature. The body temperature of the animals will affect their activity, so this information can be relevant for comparing between sampling periods in a monitoring activity. For measuring the body temperature we can use cloacal thermometers or digital thermometers with a probe. Take into consideration that, especially for smaller specimens, contact with our hands will affect their body temperature, so the measuring of temperature should be done immediately upon capturing the animal.

After collecting the animals it can sometimes be necessary to preserve them as vouchers. The preservation of specimens is a key element for taxonomic identification and when accompanied by properly compiled field notes, it becomes an excellent resource for scientific research in many branches of biology. For example, historical data from museum specimens can allow researchers to detect and assess changes in biodiversity in an area over time. For the preparation of vouchers it will be necessary to kill the animals, although in some cases it is possible to use animals that are already dead due to traps or road mortality. We should collect the minimum number of specimens possible

depending on the aims of our study. Although it can depend on how common the animal in question is, it would be advisable to preserve around 20-30 animals for scientific studies and a minimum of 4 for voucher specimens (Graeter *et al.*, 2008). It is mandatory to follow any institutional guidelines that may apply or to request the necessary permits. The procedure to euthanize the sampled animals should be humane and should preserve the condition of the animal. The most preferred techniques for killing reptiles and amphibians are by injecting or submerging the animals in lethal doses of one of the following:

- Sodium pentobarbital
- Hydrous chlorobutanol
- Tricaine methenesulfonate
- Cloretone
- Ethanol
- Other anesthetics

In the case of amphibians, due to their permeable skin, immersion in anaesthetic solutions is the most frequent way of humanely killing them. The most common products used are chlorobuthanol and tricaine methanesulfonate, also called MS-222 (Andreone *et al*., 2008). The minimum concentration should be 250 mg/l (concentrations >500 mg/l must be buffered with an equal weight of sodium bicarbonate as it is an acidic product).

In the case of reptiles, sodium pentobarbital has traditionally been used injected intravenously, intra-abdominally or intrapleuropitoneally (Cooper *et al*., 1989), but recently the use of MS-222 has also been recommended through intracoleomic injections of 250 to 500 mg/kg at 1% solution (Conroy *et al.,* 2009).

The fixation of the specimens should only begin once we are sure that the animals are dead. As chemical fixation affects the proteins in the tissue of the animals, we should attempt to fix them in positions that preserve their morphology and that allows for the observation of key identification characters. The fixation in 10% formalin (obtained by diluting 40% formol) allows a better preservation of morphology so it is ideal for the animals that will be used for formal taxonomic description or for exhibit. Formalin is carcinogenic, flammable and dangerous if fumes are inhaled, so the appropriate cautions must be taken when working with it. In addition it will not allow to use the specimens for posterior DNA analysis so it is advisable to collect tissue samples before fixing the entire specimen, and fix these in pure ethanol. In case formalin is not available, 70% ethyl alcohol can be used, but other alcohols are not recommended (McDiarmid, 1994).

Once the specimen is fixed, it is extremely important to attach each specimen with data such as the field number and any information recorded from the field (GPS coordinates, time, habitat, initial identification, collector, sampling method or weather conditions). It is advisable to use acronyms in the field number referring to the collector, followed by a progressive number and keep the same structure within sampling efforts. This should be printed in hard paper resistant to ethanol and formalin; either hand-written or printed with water resistant ink as there is a risk of loosing the information during transport or long-term storage.

Dependent of the aim of the study it could only be necessary to take a tissue sample or biopsy from the captured animals instead of preserving the whole specimen.

Blood samples are the most common procedures as when it is correctly done it may be less invasive than taking other tissues. In the case of DNA analysis rather small amounts of blood will be necessary, although the amount will be larger for physiological studies. Turtle blood can be obtained from a femoral or jugular vein, a carotid artery, the retrorbital space or the paired cervical sinuses (Dessauer, 1970). In medium and large sized lizards blood is typically collected from orbital sinuses (*e.g.*, Haenel *et al*., 2003), and in crocodilians blood is normally taken from internal jugular or caudal veins. In the case of amphibians, only the larger species can endure blood sampling and this can be done through the midline abdominal vein. Finally for most relatively large reptiles and amphibians heart puncture can also be a viable way to extract blood although this can cause mortality if done by inexperienced workers. In the case of smaller animals, heart puncture will be the only way to take blood samples and will necessarily be fatal. The blood samples can be collected through heparinized capillary tubes.

Alternative tissue samples that can be collected in reptiles and amphibians are tail clips from salamanders, lizards, turtles or snakes. Toe clips may be used as well in salamanders, frogs and lizards, while clipping scutes of the tail of crocodilians and ventral scales from snakes are also common practice. These sampling techniques have the additional benefit of potentially being very useful to researchers who need to mark animals for individual identification.

Finally for DNA studies there is the possibility of using other non traditional sources of tissue which are not aggressive but can later prove difficult to analyze due to the low molecular weight and concentration of DNA in the samples. The most relevant of these sources in amphibians and reptiles are feces, although orifice swabs and shell or scale remnants can also be useful (Poschadel & Moller, 2004). For methods to better preserve tissues for future DNA analyses, we refer to the chapter of Gemeinholzer *et al*. (this volume) on organizing specimen and tissue preservation techniques in the field for subsequent molecular analyses

5.2. Photo-vouchering

Photo-vouchering entails using photographs to document the occurrence of encountered wildlife. This is particularly useful in herpetology as it is very possible to make photographs of the animals accenting the key features that allow for a doubtless identification. These photo-vouchers, if correctly complemented with additional information will provide long-term evidence that those species exist or existed in a given geographical location. A literature record complemented with a photograph will make the report reliable without the shadow of a doubt. In addition photo-vouchers can be the alternative to traditional vouchers in the case of rare, threatened and endangered species or the alternative to the records of animals difficult to capture, such as basking water turtles. In the cases when the preparation of vouchers specimens is

absolutely unavoidable, photographs of the living animal will also be of much help as after fixation specimens tend to lose their colours and even some patterns. The ideal situation of documenting the occurrence of a certain species is having the voucher specimen for detailed analysis complemented with photographs of the specimen before fixating.

Currently the use of digital cameras has made photographing cheaper. It is possible to quickly review the photographs taken and make as many pictures as necessary, although we should remember that digital files can also become corrupted and the information lost. Some recommendations for preparing photo-vouchers are:

- If the photographs are going to eventually be deposited in a natural history museum or other repositories we should obtain information on the format, size and resolution needed;
- Include some kind of scale in the photograph to have information on the size of the animal photographed;
- Make the photographs of the animals as soon as possible after capturing, as especially some amphibians tend to change colours and patterns after being captured;
- If the animals are very active, it can be useful to lightly chill them in a refrigerator, but never in the freezer. The amount of time should never be over a few minutes depending on the size, and if the animals are later going to be released back to the wild, first make sure that it has returned to normal temperature before doing so.

6. Field notes and data collection

Most serious shortfalls in gathering and managing descriptive data on amphibians and reptiles can be avoided through planning and preparation prior to collecting data. The list below compiled by Greene (2008) includes common issues and problems that need to be addressed when implementing an inventory or monitoring program:

- Research and study goals and the specific data to be gathered must be clear to all parties involved (*e.g.*, funding agency representatives, researchers, and technicians);
- Data must be gathered in an organized, consistent manner. Design a datasheet that is objective and simple to use, and which includes all relevant information in sufficient detail. If funds and expertise allow it, invest in personal digital assistants (PDAs) or electronic laboratory notebooks which can be programmed with customized forms for direct data entry in the field (this can help minimize data entry and data transfer errors);
- All personnel involved must be trained to gather data in the same manner. Attention to detail and consistency are paramount. Handwriting must be legible;
- Store data routinely in one place until the data can be entered into a database. Keep electronic backups or photocopies of the originals in a different secure location. More than one person should be familiar with the procedure and storage locations;

- Consider how the data will be used and then enter the data into an appropriately designed database. A spreadsheet such as Microsoft-Excel is adequate for many straightforward datasets. Microsoft-Access may be a better option if the data are a subset of a bigger relational database. Copy the data on a weekly basis at minimum to a portable storage medium and keep the files in a separate location;
- Review the data and the data management system early in the process and then periodically on a regular basis. This will allow early detection of errors and inconsistencies, which can be identified and corrected before valuable information is lost;
- One competent, detail-oriented person should oversee the entire process from data collection to data entry to data storage.

For some examples of datasheets that can be used during inventories and monitoring, I refer to Graeter (2008).

7. Acknowledgements

I would like to thank Franco Andreone and José Pedro Sousa do Amaral who reviewed parts of the text. Andreas Schlüter kindly read the whole manuscript and gave comments and suggestions to improve it. I am also in debt to an anonymous referee that helped to greatly improve the final structure and contents of the chapter. Finally I would like to thank Abc Taxa editor Yves Samyn and Jutta Eymann for their enormous help throughout the editorial process.

8. References

ALFORD, R.A. 1986. Habitat use and positional behavior of anuran larvae in a northern Florida temporary pond. *Copeia* 1986: 408-423.

ANDREONE, F., RANDRIAMAHAZO, H. & RABIBISOA, N.H.C. 2008. Standardisation in conservation activities. *In*: ANDREONE, F. & RANDRIAMAHAZO H. (Eds). *Sahonagasy Action Plan. Conservation Programs for the Amphibians of Madagascar / Programmes de Conservation pour les Amphibiens de Madagascar*. Museo Regionale di Scienze Naturali, Conservation International, IUCN / Amphibian Specialist Group: 84-94.

ANDREWS, M.A. 2008. Road cruising. *In:* GRAETER, G.J., BUHLMANN, K.A., WILKINSON, L.R. & GIBBONS, J.W. (Eds). *Inventory and Monitoring: Recommended Techniques for Reptiles and Amphibians, with application to the United States and Canada*. PARC Technical Report. Aiken, South Carolina: 119-124.

BLAUSTEIN, A.R., WAKE D.B. & SOUSA, W.P. 1994. Amphibian Declines: Judging Stability, Persistence, and Susceptibility of Populations to Local and Global Extinctions Source. *Conservation Biology* 8: 60-71.

BROWN, G.W. 2001. The influence of habitat disturbance on reptiles in a Box-Ironbark eucalypt forest of south-eastern Australia. *Biodiversity and Conservation* 10: 161-176.

BURGDORF, S.J., RUDOLPH, D.C., CONNER, R.N., SAENZ, D. & SCHAEFER, R.R. 2005. A successful trap design for capturing large terrestrial snakes. *Herpetological Review* 36: 421-424.

BUHLMANN, K.A. & VAUGHAN, M.R. 1991. Ecology of the turtle *Pseudemys concinna* in the New River, West Virginia. *Journal of Herpetology* 25: 72-78.

CONROY, C.J.T, PAPENFUSS, T., PARKER, J. & HAHN, N.E. 2009. Use of Tricaine Methanesulfonate (MS222) for Euthanasia of Reptiles. *Journal of the American Association for Laboratory Animal Science* 48: 28-32

COOPER, J.E., EWBANK, R., PLATT, C. & WARWICK. C. 1989. *Euthanasia of Amphibians and Reptiles*. University Federation for Animal Welfare, Potters Bar: 35 pp.

CORN, P.S. 1994. Straight-line drift fences and pitfall traps. *In* HEYER, W.R., DONNELLY, M.A., MCDIARMID, R.W., HAYEK, L.C. & FOSTER M.S. (Eds). *Measuring and monitoring biological diversity: standard methods for amphibians*. Smithsonian Institution Press, Washington DC: 107-119.

CRUMP, M. L., & SCOTT, JR., N.J. 1994. Visual encounter surveys. *In* HEYER, W.R., DONNELLY, M.A., MCDIARMID, R.W., HAYEK, L.C. & FOSTER M.S. (Eds). *Measuring and monitoring biological diversity: standard methods for amphibians*. Smithsonian Institution Press, Washington DC: 84-92.

DALY, J.W. 1998. Thirty Years of Discovering Arthropod Alkaloids in Amphibian Skin. *Journal of Natural Products* 61: 162-172.

DESSAUER, H.C. 1970. Blood chemistry of reptiles: physiological and evolutionary aspects. In GANS, C. & PARSONS, T.S. (Eds). *Biology of the Reptilia. Vol. 3*. Academic Press, New York: 1-72.

DODD, C.K., Jr. 2003. *Monitoring amphibians in Great Smoky Mountains National Park*. U.S.Geological Survey Circular 1258, Tallahassee, FL: 117 pp.

DODD, C.K., Jr. 2009. *Ecology and Conservation of Amphibians. A Handbook of Techniques*. Oxford University Press: 464 pp.

DODD, C.K., JR., & SCOTT D.E. 1994. Drift fences encircling breeding sites. *In*: HEYER, W.R., DONNELLY, M.A., MCDIARMID, R.W., HAYEK, L.C. & FOSTER M.S. (Eds). *Measuring and monitoring biological diversity: standard methods for amphibians*. Smithsonian Institution Press, Washington DC: 125-130.

ENGE, K.M. 2001. The pitfalls of pitfall traps. *Journal of Herpetology* 35: 467-478.

FITCH, H.S. 1987. Collecting and life-history techniques. *In*: SEIGEL, R.A., COLLINS, J.T. & NOVAK, S.S. (Eds). *Snakes: Ecology and Evolutionary Biology*. Macmillan, New York: 143-164.

FITCH, H.S. 1992. Methods of sampling snake populations and their relative success. *Herpetological Review* 23: 17-19.

GIBBONS, J.W., SCOTT, D.E., RYAN, T.J., BUHLMANN, K.A., TUBERVILLE, T.D., METTS, B.S., GREENE, J.L., MILLS, T., LEIDEN, Y, POPPY, S. & WINNE, C.T. 2000. The global decline of reptiles, déjà vu amphibians. *Bioscience* 50: 653-666.

GLAUDAS, X.A. 2008. Aerial surveys. *In:* GRAETER, G.J., BUHLMANN, K.A., WILKINSON, L.R. & GIBBONS, J.W. (Eds). *Inventory and Monitoring: Recommended Techniques for Reptiles and Amphibians, with application to the United States and Canada*. PARC Technical Report. Aiken, South Carolina: 125.

GRAETER, G.J. 2008. Appendix VIII. Sample datasheets. *In*: GRAETER, G.J., BUHLMANN, K.A., WILKINSON, L.R. & GIBBONS, J.W. (Eds). *Inventory and Monitoring: Recommended Techniques for Reptiles and Amphibians, with application to the United States and Canada*. PARC Technical Report, Aiken, South Carolina: 215-231.

GRAETER, G.J., BUHLMANN, K.A., WILKINSON L.R. & GIBBONS J.W. 2008. *Inventory and Monitoring: Recommended Techniques for Reptiles and Amphibians, with application to the United States and Canada*. PARC Technical Report, Aiken, South Carolina: 301 pp.

GREENBERG, C.H., NEARY, D.G. & HARRIS, L.D. 1994. A comparison of herpetofaunal sampling effectiveness of pitfall, single-ended, and double-ended funnel traps used with drift fences. *Journal of Herpetology* 28(3): 319-324.

GREENE, J.L. 2008. Standards and data management. *In:* GRAETER, G.J., BUHLMANN, K.A., WILKINSON, L.R. & GIBBONS, J.W. (Eds). 2008. *Inventory and Monitoring: Recommended Techniques for Reptiles and Amphibians, with application to the United States and Canada*. PARC Technical Report, Aiken, South Carolina: 71-72.

HAENEL, G.J., SMITH, L.C. & JOHN-ALDER, H.B. 2003. Home-range analysis in *Sceloporus undulatus*. II. A test of spatial relationships and reproductive success. *Copeia* 2003: 113-123.

HANLIN, H.G., MARTIN, F.D., WIKE, L.D. & BENNETT, S.H. 2000. Terrestrial activity, abundance and species richness of amphibians in managed forests in South Carolina. *American Midland Naturalist* 143(1): 70-83.

HARRIS, R.N., ALFORD, R.A. & WILBUR, H.M. 1988. Density and phenology of *Notophthalmus viridescens dorsalis* in a natural pond. *Herpetologica* 44: 234-242.

HEYER, W.R., DONNELLY, M.A., MCDIARMID, R.W., HAYEK, L.C. & FOSTER, M.S. 1994. *Measuring and monitoring biological diversity: standard methods for amphibians*. Smithsonian Institution Press, Washington, DC: 364 pp.

HOUZE, C.M. JR., & CHANDLER, C.R. 2002. Evaluation of coverboards for sampling terrestrial salamanders in South Georgia. *Journal of Herpetology* 36(1): 75-81.

JAEGER, R.G. 1994a. Patch sampling. *In*: HEYER, W.R., DONNELLY, M.A., MCDIARMID, R.W., HAYEK, L.C. & FOSTER M.S. (Eds). *Measuring and monitoring biological diversity: standard methods for amphibians*. Smithsonian Institution Press, Washington DC: 107-109.

JAEGER, R.G. 1994b. Standard techniques for inventory and monitoring: transect sampling. *In*: HEYER, W.R., DONNELLY, M.A., MCDIARMID, R.W., HAYEK, L.C. & FOSTER M.S. (Eds). *Measuring and monitoring biological diversity: standard*

methods for amphibians. Smithsonian Institution Press, Washington DC: 103-107.

JAEGER, R.G. & INGER, R.F. 1994. Quadrat sampling. *In*: HEYER, W.R., DONNELLY, M.A., MCDIARMID, R.W., HAYEK, L.C. & FOSTER M.S. (Eds). *Measuring and monitoring biological diversity: standard methods for amphibians*. Smithsonian Institution Press, Washington DC: 97-102.

KOK, P.J.R. & KALAMANDEEN, M. 2008. Introduction to the taxonomy of Kaieteur National Park, Guyana. *ABC Tax,* 5: 278 pp.

LINDEMAN, P.V. 1998. Of deadwood and map turtles (*Graptemys*): An analysis of species status for five species in three river drainages using replicated spotting-scope counts of basking turtles. *Chelonian Conservation and Biology* 3(1): 137-141.

MARCELLINI D.L. & JENSSEN, T.A.. 1991 Avoidance Learning by the Curly-Tailed Lizard, *Leiocephalus schreibersi*: Implications for Anti-Predator Behavior. *Journal of Herpetology* 25(2): 238-241.

MCDIARMID, R.W. 1994. Preparing amphibians as scientific specimens. *In*: HEYER, W.R., DONNELLY, M.A., MCDIARMID, R.W., HAYEK, L.C. & FOSTER M.S. (Eds). *Measuring and monitoring biological diversity: standard methods for amphibians*. Smithsonian Institution Press, Washington DC: 289-297 .

MEIK, J.M., JEO, R.M., MENDELSON III, J.R. & JENKS, K.E. 2002. Effects of bush encroachment on an assemblage of diurnal lizard species in central Namibia. *Biological Conservation* 106: 29-36.

MILLS, M.S., HUDSON, C.J. & BERNA, H.J. 1995. Spatial ecology and movements of the brown water snake (*Nerodia taxipilota*). *Herpetologica* 51: 412-423.

MITCHELL, J.C. 2000. *Amphibian Monitoring Methods and Field Guide*. Smithsonian National Zoological Park, Conservation Research Center, Front Royal, VA: 56 pp.

MOURÃO, G., COUTINHO, M., MAURO, R., CAMPOS, Z., TOMAS, W. & MAGNUSSON, W. 2000. Aerial surveys of caiman, marsh deer, and pampas deer in the Pantanal wetland of Brazil. *Biological Conservation* 92: 175-183.

NELSON, D.H. & GIBBONS, J.W. 1972. Ecology, abundance, and seasonal activity of the scarlet snake, *Cemophora coccinea*. *Copeia* 1972: 582-584.

PARMALEE, J.R. & FITCH, H.S. 1995. An experiment with artificial shelters for snakes: effect of material, age, and surface preparation. *Herpetological Natural History* 3: 187-191.

PAULEY, T.K. & LITTLE, M. 1998. A new technique to monitor larval and juvenile salamanders in stream habitats. *Banisteria* 12: 32-36.

PETERSON, R.C. & CUMMINS K. W, 1974. Leaf processing in a woodland stream. *Freshwater Biology* 4: 343-368.

POSCHADEL, J.R. & MOLLER, D. 2004. A versatile field method for tissue sampling on small reptiles and amphibians, applied to pond turtles, newts, frogs, and toads. *Conservation Genetics* 5: 865-867.

RODDA, G.H. & DEAN-BRADLEY, K. 2002. Excess density compensation of island herpetofaunal assemblages. *Journal of Biogeography* 29: 623-632.

RUSSELL, K.R., HANLIN, H.G., WIGLEY, T.B. & GUYNN, D.C. 2002. Responses of isolated wetland herpetofauna to upland forest management. *Journal of Wildlife Management* 66: 603-617.

RYAN, T.J., PHILIPPI, T., LEIDEN, Y.A., DORCAS, M.E., WIGLEY, T.B. & GIBBONS, J.W. 2002. Monitoring herpetofauna in a managed forest landscape: effects of habitat types and census techniques. *Forest Ecology and Management* 167: 83-90.

SEMLITSCH, R.D. 2003. *Amphibian Conservation*. Smithsonian Books, Washington DC: 324 pp.

SEMLITSCH, R.D., BROWN, K.L. & CALDWELL, J.P. 1981. Habitat utilization, seasonal activity, and population size structure of the southeastern crowned snake *Tantilla coronata*. *Herpetologica* 37: 40-46.

SHAFFER, H.B., ALFORD, R.A., WOODWARD, B.D., RICHARDS, S.J., ALTIG, R.G. & GASCON, C. 1994. Quantitative sampling of amphibian larvae. *In*: HEYER, W.R., DONNELLY, M.A., MCDIARMID, R.W., HAYEK, L.C. & FOSTER M.S. (Eds). *Measuring and monitoring biological diversity: standard methods for amphibians*. Smithsonian Institution Press, Washington DC: 130-141.

SKELLY, D.K. 1996. Pond drying, predators, and the distribution of *Pseudacris* tadpoles. *Copeia* 1996: 599-605.

SMITH, L.L., BARICHIVICH, W.J., STAIGER, J.S., SMITH, K.G. & DODD, JR., C.K. 2006. Detection probabilities and occupancy estimates for amphibians at Okefenokee National Wildlife Refuge. *American Midland Naturalist* 155: 149-161.

SUTTON, P.E., MUSHINSKY, H.R. & MCCOY, E.D. 1999. Comparing the use of pitfall drift fences and cover boards for sampling the threatened sand skink (*Neoseps reynoldsi*). *Herpetological Review* 30: 149-151.

TODD, B.D., WINNE, C.T., WILLSON, J.D. & GIBBONS, J.W. 2008. Getting the drift: effects of timing, trap type, and taxon on herpetofaunal drift fence surveys. *American Midland Naturalist* 158: 292-305.

VOGT, R.C. 1980. New methods for trapping aquatic turtles. *Copeia* 1980: 368-371.

WALDRON, J.L., DODD, C.K. & CORSER, J.D. 2003. Leaf litterbags: Factors affecting capture of stream-dwelling salamanders. *Applied Herpetology* 1: 23-36.

WILLSON, J.D. & DORCAS, M.E. 2003. Quantitative sampling of stream salamanders: comparison of dipnetting and funnel trapping techniques. *Herpetological Review* 34: 128-130.

Chapter 21

Tips for bird surveys and censuses in countries without existing monitoring schemes

by

Friederike Woog
State Museum of Natural History Stuttgart
Rosenstein 1, 70191 Stuttgart, Germany
Email: woog.smns@naturkundemuseum-bw.de

Swen C. Renner
Institute of Experimental Ecology, University of Ulm
Albert-Einstein Allee 11, 89069 Ulm, Germany
Email: swen.renner@uni-ulm.de

Jon Fjeldså
Zoological Museum, University of Copenhagen
Universitetsparken 15, DK-2100 Copenhagen, Denmark
Email: JFjeldsaa@snm.ku.dk

Abstract

Birds are important environmental indicators and, for a long time, have been included when surveying biodiversity. This has led to a whole array of publications, some of which are available over the Internet, making them easily accessible worldwide. Here we provide practical guidance, with relevant source references, for how to plan and conduct bird surveys and censuses, especially in tropical environments.

Keywords: biodiversity, bird counts, assessments, monitoring techniques

1. Introduction

When surveying biodiversity, birds are usually included because they have been more completely charted (in terms of taxonomy and distributions) than any other taxonomic group, and because good field guides, and even bioacoustics data, are available for identification for most parts of the world. Furthermore, a large number of skilled birdwatchers are often keen to volunteer in bird monitoring projects. In order to develop bird monitoring as an effective tool in conservation biology, a whole array of literature about bird census and monitoring techniques has been published, the standard book by the late Bibby and his colleagues (2000) covering most of them.

In 1998, Bibby *et al.* presented a guide especially designed for expeditions. Gilbert and a team of specialists published a comprehensive book on monitoring techniques for all sorts of UK bird species ranging from songbirds to raptors to waterfowl (Gilbert *et al.*, 1998). A "Best Practice Guide for Wild Bird Monitoring" was published in 2008 by Voříšek and his colleagues focusing mainly on Europe and giving an overview of existing monitoring schemes. It can be downloaded, making it easily accessible to birdwatchers and ornithologists worldwide (http://www.ebcc.info/index.php?ID=365). More articles with information on specialized count procedures can be found in Gibbons & Gregory (2006). So why include birds in this manual? The goal of this article is to give practical advice on how to plan bird surveys and censuses in countries where monitoring schemes are lacking, and to provide useful Internet links. This is by no means a complete treatment of methods, study design, data management and analysis as this would be far beyond the scope of this chapter. All of these, sometimes rather complicated topics, are covered well in the publications mentioned above (also see Gregory *et al.*, 2004).

This chapter is written from a European perspective. Whereas the general biology and life cycle of European and North American birds is rather well known, we know far less about birds from many other parts of the world. It is useful and important that scientists from developed countries contribute to the study of biodiversity outside Europe, especially in the tropics. To be sustainable, the long-term monitoring in developing countries should be locally based (Danielsen *et al.*, 2006, 2007). Although hard to achieve, this goal should always be kept in mind, and is indeed often feasible once local communities experience how simple monitoring systems can be used proactively to manage their own resources.

2. Preparation for the survey

To start with, we advise contacting local ornithologists, to tell them about the plans and to ask them if they support the idea of a survey and if they would become a partner. It is important to find the right people to work with, people that are accepted locally but ideally biologists by training. In most countries birdwatchers and ornithologists are associated with the Birdlife International Partner and can be tracked down by visiting the Birdlife International homepage, a network of birdwatchers and ornithologists worldwide :

http://www.birdlife.org/worldwide/national/index.html. A local partner can help to answer the following questions: (1) Have surveys already been carried out in the target area or are some being planned? (2) Do other monitoring schemes already exist? (3) When is the best time for a survey (season and time of day)? (4) Are permits needed and how are they to be obtained? If a certain survey or monitoring scheme is already in place one should consider choosing a similar method to make data comparable between sites. A good example for a large-scale bird survey is the second South African Bird Atlas Project SABAP2 (http://sabap2.adu.org.za/index.php). On the homepage there are good descriptions of survey methods and databasing procedures including various downloads.

For your partnership to work, consider that volunteer schemes, as developed in Europe and South Africa (breeding bird surveys, international waterfowl counts etc.) rarely exist in developing countries. People have to work to survive and can rarely afford a hobby like birding. Therefore, find out what the “normal” fees and salaries are. Discuss this issue with your local partner beforehand. Out in the villages, it may be useful to make agreements about donations and salaries with a village chairman, or council, rather than with the individual helpers. Such discussions may be cumbersome (for you) and you may feel awkward, but nothing is worse than having to sort out conflicts afterwards. Sometimes it can be useful to have a small contract telling the nature of your cooperation who is responsible for which aspect, signed by all parties involved and a copy resting with each party.

2.1. Species identification

When planning a survey in an unfamiliar region, prepare yourself beforehand, as this will save a lot of time in the field. This includes surveying existing ornithological literature about birds in the target area, and to identify species of particular interest. For most parts of the world there are field guides for birds and CDs with bird calls and songs. The quality of these guides greatly varies and they rarely include juvenile birds. Some are heavy to take into the field. A simple although somewhat drastic trick is to ask a book binder to split your book into two – one with the plates (to bring into the field) and one with the more extensive text that you may decide to leave at home or at base camp. Some publishers have already caught up with this idea, *i.e.* for West Africa (Borrow & Demey, 2004), New Zealand (Robertson & Heather, 2004) or South Asia (Rasmussen & Anderton, 2005). A good source to check what species occur in an area is http://www.birdtours.co.uk/ which is a collection of trip reports by travelling birders all around the world, including up to date maps, tips on where to stay and who the useful local contacts are.

2.2. Calls and Songs

We very much recommend using a MP3 player with headphones and microphone that can easily be taken into the field. With a special amplifier, a directional microphone can be used, increasing the range and quality of the recordings. The calls from a CD can be transferred onto the player. Most

modern recordings will offer files in mpg-format. If not, the sound files on audio CDs can be transcribed into mpg-files easily, using freeware available (*i.e*. http://www.freerip.com/). Do observe copyright laws and make sure your download is really for free. Free resources are online sound libraries:
http://www.xeno-canto.org/africa/index_static.html
http://www.xenocanto.org/index_static.html
http://www.xeno-canto.org/asia/index_static.html
http://www.xeno6canto.org/australasia/index_static.html

If you are after species from an area where sound recordings are not yet available, you can check with the Wildlife Section of the British Library National Sound Archive (NSA, http://www.bl.uk/soundarchive) or the Library of Natural Sounds (LNS) at Cornell Laboratory of Ornithology (www.birds.cornell.edu). Fees may apply. Once you have all songs and calls you need, arranging the files in folders is useful, so that they can be easily found when in the field (*i.e.* in alphabetical or systematic order, whatever the preference). Time permitting, calls of species one expects in the target area can be put into a separate folder. The recording function of most players is usually good enough to make a (low quality) recording of a bird call or song that cannot be identified at once. These can later be sent to a specialist to aid identification. Take a player with regular AA or AAA batteries as those usually can be bought in most countries or, when sunny enough, use small solar battery rechargers. Avoid complicated recharging systems that you need electrical power and adaptors for, as electrical power may not be available at base camp. In the headphone slot one can usually plug small active speakers (working with batteries) that can be used for playback. We do not recommend the use of playback but for certain species it may be necessary, especially to detect cryptic or understorey species. Playback can disturb birds, especially in the breeding grounds. Therefore it should be only used if absolutely necessary and then only very briefly, *i.e.* for a maximum of five minutes. As soon as there is a reaction, stop. Keep in mind that a bird may not visibly respond, yet may still be disturbed.

2.3. Bird collections in museums

Bird collections are good places to brush up bird identification skills before going into the field. If a survey is for scientific and conservation purposes, most museums will allow such studies. See for example:
http://www.museum.lsu.edu/~Remsen/AVECOLlections.html
http://www.scricciolo.com/European_Bird_Collections_C%20S%20Roselaar.pdf

To find out whether a museum holds the required specimens, the bird curator should be contacted well in advance and arrangements made to see specimens. Bird curators or collection staff should explain the best way how to handle bird skins. Always handle them with great care because they are meant to be used by generations to come! A large number of web pages are useful to study birds. Many of them are accessible through the GBIF platform (http://www.gbif.org/). A useful searchable database is http://avibase.bsc-eoc.org giving links to selected Google images, distribution maps, taxonomy, ITIS, Birdlife and Wikipedia. ORNIS, the Ornithological Information System, is linked to GBIF and allows searching 42 mostly American bird databases, including museum specimens (http://olla.berkeley.edu/ornisnet/). The site offers

a list of the respective curators including email addresses. For the Neotropics, another good source is http://neotropical.birds.cornell.edu/portal/home. The Zoological Museum, University of Copenhagen offers online access to birds collected in Tanzania (http://www.zmuc.dk/VerWeb/Tanzanian_Vertebrates/TanzVert.index.html).

2.4. When to count

The timing of a bird survey will depend on the life cycle of a particular bird species if monitoring one species. Of course, many surveys would be for more than one species. To get an inventory for a given area, typically the major breeding season is best suitable for monitoring the community. Some birds migrate and will be absent from the chosen study area for part of the year. This can include a smaller scale *i.e.* altitudinal movement, but can range to long-distance migration. For most surveys, the time when males are singing on their territory and the birds constructing their nests, are the most suitable, since birds are most active then. Once sitting on the nest, birds often become very silent and cryptic, making it hard for anybody to detect them. In many tropical areas breeding seasons are not as synchronized as in more temperate regions. This means that often only some bird species are breeding whereas other, often closely related species may breed much later. Furthermore, only some individuals within a population may be breeding. The timing of breeding greatly depends on the altitude of your study site and the weather. Even rainforests can be rather dry in certain years. Humidity greatly affects food availability and triggers the onset of the breeding season. Low temperatures in mountain regions can defer the onset of the breeding season. It is often very hard to predict these patterns, even for experienced local people. If logistically possible, we recommend to count at least two times a year.

2.5. Target species

You should aim to find all species possible including breeding birds, wintering birds, cryptic species, nocturnal species, understorey species, rare species, bird colonies and mixed species flocks. Special methods are available for most or can be adapted from closely related species (see Gilbert *et al.*, 1998). The more time you spend in an area, the more species you are likely to find. These species discovery curves (Fig. 1) are quite useful as they help you to identify the point in time when the number of new species discovered in an area becomes rather low. For economic reasons and depending on the question being investigated, one may decide to stop data collection at that point (see below for further details).

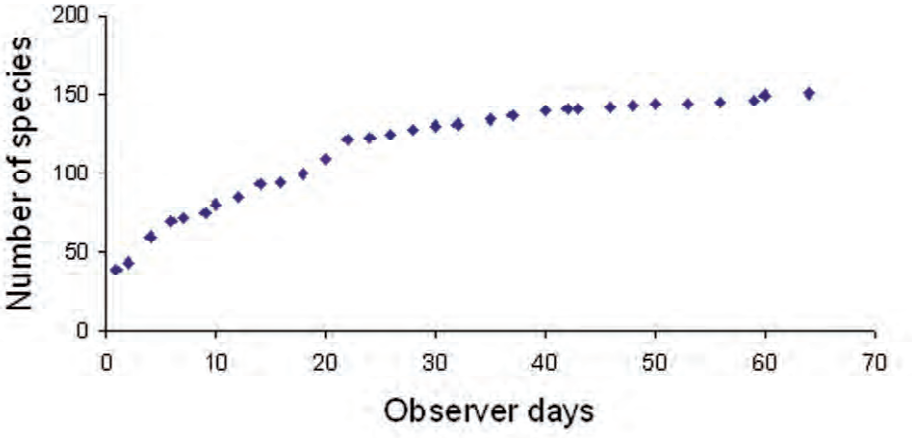


Fig. 1. Species discovery curve, with the total cumulative number of species discovered each day (the time unit could also be hours). At first, the number of species increases rapidly until, after a certain number of days, this number levels off. After that it takes many days to discover a few more species, some of which may only be occasional visitors of the area (after Bibby *et al.*, 1998).

2.6. Habitat

Birds live in almost any habitat you can think of. Clearly, you need to adapt your survey method to the habitat. As an example of two extremes, consider a desert with very low vegetation on one hand, and a dense rainforest with trees ranging up to 40 m into the sky on the other. It is therefore useful to split your survey area into different habitats (*i.e.* forest, scrub, desert, alpine etc.) and to adjust your survey method accordingly (*i.e.* spacing of transects, distance of sample points etc.). If you want to compare different habitats you should, however, use the same protocol. Bibby *et al.* (1998, 2000), Gilbert *et al.* (1998), Gregory *et al.* (2004) and Gibbons & Gregory (2006) offer good advice on this topic.

2.7. Maps

High-resolution maps are essential for a good survey and we recommend obtaining maps prior to surveys. In the capitals of most countries you can contact ministries for geography/geology or local cartographic services. Google Earth images can give you a good idea of your study area, and for many areas high-resolution images are now available. Sometimes they are a few years old and therefore of limited value in areas under rapid “development”.

2.8. Bird behaviour

Birds have very different life styles. Some spend almost all their time up in the air (*e.g.* swifts), some are flightless (*e.g.* Kakapo, Kiwi) or virtually so and skulk around in the understorey vegetation. Birds that live up in the canopy are often almost impossible to detect, as are birds that hide in dense foliage. A few birds

are rather curious and easy to detect, while others are extremely shy. Species detectability depends very much on bird behaviour but also on weather conditions and the skill of the observer. Observers who recognise all of the bird calls and songs of an area will naturally discover more bird species than observers without these skills.

Birds also sing at very different times of day, some start very early in the morning, or advertise their territory around daybreak by giving a single call, some sing at night (*e.g.* owls). Whereas for some species it may be useful to count breeding territories identified by their territorial song, for other species it may work better to count them during foraging or when they are flying to a roost (*i.e.* gulls or terns). Many tropical forest birds move around in multi-species feeding parties, or become active only when such parties pass through their territory and provide effective antipredator vigilance. Thus, it is of great advantage to pay attention to these bird parties, which often follow the same route day after day (Poulsen, 1996). In the neotropics, mixed flocks are known to follow ant swarms (Vallely, 2001; Roberts *et al.*, 2000).

Although the books by Bibby *et al.* (1998, 2000) and Gilbert *et al.* (1998) offer more detailed guidance, the annual cycles of birds outside Europe and North America are, in comparison, poorly known. Indigenous people are often the only ones that can give you some ideas about certain species (Ng'weno, 2008). Sometimes their stories may make Europeans sneer because they contain a lot of mystery. Don't sneer at the stories but try to interpret them. A bird spending winter in a tree cavity and coming out of its hole when the thunder arrives could simply mean that it is a migratory species returning with the rains. Documenting all bird behaviour and observations during surveys is a very worthwhile exercise!

2.9. Local knowledge and training of locals

Once in the field, it is very useful to ask a local guide, hunter or project partner to accompany you and to talk to local communities. Going through a bird book with indigenous people can give you priceless information. Depending on where in the world you are, birds are often part of the day-to-day diet of people, so they may have extensive knowledge about them. Talking to the elders of a village one may also find out which species used to occur in an area but may now have become scarce or have disappeared (Ng'weno, 2008). However, when it comes to smaller, similar-looking species, locals often cannot distinguish them as they lack binoculars. It is useful to bring extra binoculars that you can give to local guides or other project participants. To get hold of them ask friends and colleagues back home if they have a pair they don't need anymore. The most important achievement of your trip may not be your species list, but instead the training of locals in bird identification, to make them interested and to possibly teach them how to carry out a monitoring scheme themselves (http://monitoringmatters.org, http://www.springerlink.com/content/100125/) (Danielsen *et al.* 2005, 2006). Like other monitoring approaches, locally-based methods may be less precise and biased, but may on the other hand be very effective tools for locally based resource management, once locals realize how data can be used for empowerment, *e.g.* for rapid management decisions to counter habitat loss by

interventions from foreigners and from corrupt administrations. Typically, data collected by locals may lead to prompt and local decisions, while data collected by scientists feed into long-term government regulation (Danielsen *et al.*, 2007). Thus, a combination of both is needed.

2.10. Personal safety

In remote areas always take somebody with you. Local people often know an area very well and have a fabulous sense of orientation. Put an emergency mechanism in place in case you do not return in time. Let other people know where you are going and how long for. When discovering a rare animal the temptation to leave known ground becomes very high and suddenly you don't know how to get back. If one person remains on a path, the second person can go off in search for the animal, remaining in shouting distance you will always find your way back. A handheld GPS and a compass are very useful, but you need to know how to use them. Familiarize yourself with these on known ground. It is good to note the direction of larger roads, or rivers, mountain ranges, steep valleys as they can lead you back in case you get lost. Always think about the basics: enough water, emergency food, sun and mosquito protection, small headlamp (LED's), raingear if needed, small first aid kit, waterproof matches etc. A very powerful yet lightweight torch is the Supernova run with LED's which can function as a signal light (or to be used as a spotlight to see owls…).

Before your trip make sure you have all necessary vaccinations. When mist-netting this includes one against rabies, as bats that are sometimes caught are known to have transmitted this disease. Make sure you have enough medication for all likely diseases with you. When leaving medication behind give it to a local doctor or hospital. Being in remote places it is always useful to know first aid and to be able to diagnose diseases, not only concerning yourself but also the people that work with you (Werner, 1979; Merry, 1994; AAOS, 2007). If one does become sick, one should always go to a doctor. Usually, local doctors have a very good knowledge of local diseases.

3. Short overview of methods available

For anybody planning a survey we strongly recommend to thoroughly study Bibby *et al.* (1998) as it offers in depth advice on many relevant topics (http://biology.kenyon.edu/courses/biol229/fieldmanual%20birds.pdf). The best way to learn is to join a professional team for a few days, to get some first field experience and training. The Tropical Biology Organisation offers a wide range of training courses (http://www.tropicalbiology.org/).

There are methods that will give you an idea of the species present in an area (qualitative data) but not how many of them (quantitative data). When introducing the factor "time" or "space" into a simple species survey, you can very quickly improve the quality of your data (species discovery curve, encounter rates, MacKinnon index or timed species counts, see Table 1). Quantitative methods are, in general, more time consuming and require more

skill. There are point counts, line counts and even the mapping of territories. In quantitative methods often the distance between observer and bird has to be estimated (see below).

The key decisions are (from Gregory *et al.*, 2004):

- Do we want to estimate population size accurately or will an index meet our needs? In other words, are we interested in absolute or relative abundance (index)?
- Where will we undertake the survey?
- Should we cover the whole area of interest, or only sample part of it?
- If we plan to sample, how should we select the study sites?
- What geographical sampling units will we use? Mapped grid squares, forest blocks, or other parcels of land?
- What field method will we use?
- What are the recording units: individuals, singing males, breeding pairs, nests or territories?
- How will the subsequent data analysis be carried out?
- How will the results be reported and used?

You need to adapt your method to: a) the question you are asking, b) your skill, c) the time available, and d) the habitat. In open habitats, distance sampling may be easy and therefore the method of choice, but this will be hard in rainforest. Notice, though, that comparisons between habitats require that similar methods are being used.

When trying to survey a dense lowland rainforest you will quickly notice your limitations. It will be hard to see birds and to estimate distances to vocalizing birds. This makes standardised quantitative sampling difficult or even impossible (Bibby *et al.*, 2000). With the many logistic constraints during fieldwork in such environments, it is therefore important to consider how to best spend the time available. Rather than working hard to obtain perfect quantitative data from a single site, it may be better to use the time to get semi-quantitative data for several sites. This approximates random sampling of the metacommunity and, in addition, gives some information about variation across different habitats. Small samples mean that some rare species are unrecorded, and this truncation of the community (Preston, 1948) reduces the possibility to discriminate between different abundance models. However, even incomplete samples will suffice to identify dense (viable) populations of species of conservation concern, and will allow estimates of species richness.

3.1. Pilot Survey

Unless you have been there, you will not know what your survey area is really like. Plan a pilot survey of at least two weeks to a) get to know your species and the habitat, b) to try out methods and c) to practise them.

3.2. Qualitative methods, relative abundance

- **Simple species list.** All species are noted, regardless of time of day or season. The presence or absence of rare or threatened species (Birdlife International 2000, http://www.iucnredlist.org/) is the key for conservation and management of a site. The problem with simple species lists is that there is no control for observer effort. Chance observations will obviously increase with the time you spend in the field and some cryptic resident species don't show up immediately.

- **Species discovery curves**. Species discovery curves that record survey effort can be obtained by recording the time spent in the field for each observer. It is important that observers work at different areas or at different times. By also noting the date and time each species was discovered, some simple analysis becomes possible. Having separate lists for different areas may enable you to come up with further detail, *e.g.* if you split your area into degraded and natural forest, you can make a simple comparison

- **Encounter rates.** Encounter rates are calculated for each species by dividing the number of birds recorded by the number of hours spent searching, giving a figure of birds per hour for each species. When doing this separately for different habitats, more detailed information can be obtained. However, beware that encounter rates will vary with the structure of the vegetation. In dense vegetation encounter rates may be lower than in more open habitat. Encounter rates are not a substitute for true density estimates but they allow a comparison of relative abundance. Abundance categories can be scored.

  As an example (from Lowen *et al.*, 1996), for each species assume the number of individuals/100 field hours to be your value, then you could use the following abundance categories: rare (< 0.1), uncommon (0.1-2.0), frequent (2.1-10.0), common (10.1-40.0) or abundant (>40.0). If these categories do not work for your data you can of course adapt them accordingly. To avoid counting birds several times, it is important that different observers move to a starting point at some distance from base camp and plan their routes so that they are not overlapping. Birds that call loudly will be recorded more often than more quiet ones and the likelihood to record a species will depend on its state in the annual cycle.

- **MacKinnon lists** (MacKinnon & Phillips, 1993)**.** They are often used in "Rapid Assessments" (Herzog *et al.*, 2002). Make a list by recording each new species seen until you reach *e.g.* 20 species; then start again with a new list. Any one species will only be recorded once in your first list of 20, but may be recorded again in subsequent lists. Analysis of ten or more lists for a given area will give a good picture of its avifauna. Plotting the cumulative total number of species recorded against the number of lists made, this produces a species discovery curve whose steepness reflects species richness and indicates how many more species are likely to be found in an area (Fig. 2). If you are in a habitat that is species poor, you may decide to use a lower number than 20, maybe 15 or even 10 (Poulsen

et al., 1997). You need to try this out. Your speed of walking will greatly affect the kind of species you will encounter. Whilst walking slowly through the forest one will get a good number of scrub or canopy dwelling birds, but for some ground-dwelling birds such as pheasants, pittas or thrushes, moving quickly but silently through the forest will yield better results, as birds have less time to react to your approach. It is recommended to discover every bird that is active within 50 m from the transect line (Schieck, 1997) and thus it is not practically possible to achieve a constant walking speed.

If this method is used to describe community structure, there are some fundamental flaws, as some records will be single birds and others will be flocks with many individuals of the same species; fortunately this problem is not so serious in tropical forests where most species appear in pairs and family groups (2-4 individuals). The data can be much improved by writing down the number of individuals and use this raw data for the final analysis of community composition and species richness (Herzog *et al.*, 2002).

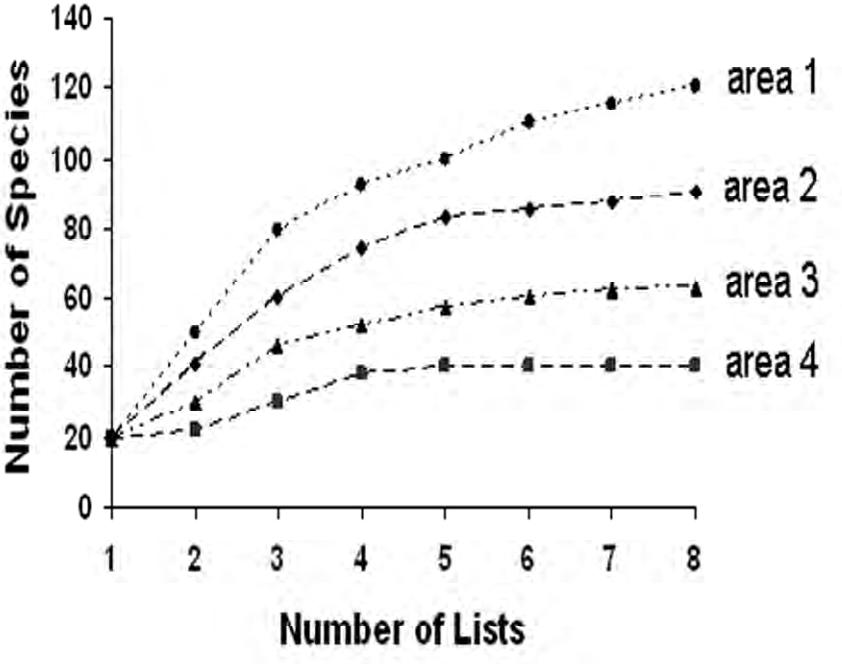


Fig. 2. Species curves derived from MacKinnon lists, simplified and altered from MacKinnon & Phillips (1993).

- **Timed species counts.** Timed species counts (TSCs) are especially useful for open habitats (Pomeroy & Tengecho, 1986), but as mentioned above this approach may not be useful off-trail in thick vegetation. Data for TSCs are recorded in six columns, corresponding to six 10-min intervals during an hour-long survey. The observer walks at a slow pace (about 1-2 km/h). For the first 10 min, every species seen or heard is noted down in the first column, regardless of the number of individuals. For the second 10 min-

period, any species not already recorded is noted in the second column and so on. For one observation hour each species is only noted once. A minimum of 15 surveys should be carried out for a site, corresponding to 15 observation hours. Pomeroy & Tengecho (1986) suggest to physically cover an area of 1 km² for each count. Depending on the habitat this may of course be modified.

3.3. Quantitative Methods

- **Positioning of sampling points.** There are various possibilities to place sampling points or beginnings of transects (Fig. 3), each one with advantages and disadvantages. For statistical reasons it is important to place all sampling points at random or at least to place your first point in a line of points at random. When counting along roads or existing paths (Fig. 3A) it is likely that not all species are discovered because the path does not run through all the different habitats. More importantly, the presence of the road or path may influence the species (or numbers) present. Placing the points randomly may give better coverage (Fig. 3B), however, choosing a completely random approach may leave some areas unsampled. A stratified random sample (Fig. 3C) using a grid (at least 500 m apart) is the best choice. In each resulting square one point is chosen at random. If points from two neighbouring squares are too close to each other (so that possibly the same birds are counted twice) then it may be useful to omit that point and choose a new one.

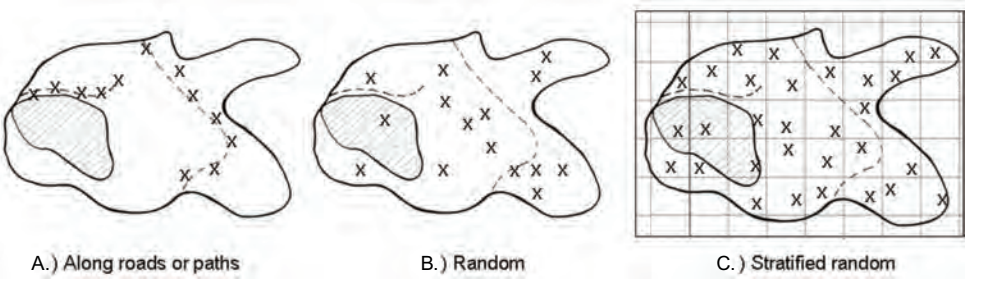


Fig. 3. Three possibilities for positioning point counts or beginnings of transects. a. along existing roads or paths; b. entirely random or c. stratified random (after Bibby *et al.*, 1998).

Method	What for?	Advantages	Disadvantages	Cost
Species list	Species present	Easy, no data analysis	No control of observer effort making comparison between areas or counts impossible	Cheap
Species discovery curve	Estimate of the total number of species present	Different sites and counts can be compared	Plotting of data requires computer analysis, but simple	
Encounter rate	Index of relative abundance for individuals of a species per unit time	Crude comparison of abundance between species within a site and within species between sites	Differences in species detectability not accounted for. To count all individuals of all species present can be a practical problem	
MacKinnon lists	Index of relative abundance based on the number of encounters with species per block of effort. Plotting a species discovery curve	Crude comparison of abundance between species within a site and within species between sites. Data collection is simple, allowing the observer freedom to roam. Relatively unaffected by observer skill and concentration	Differences in species detectability not accounted for, underestimation of flocking species	
Timed species-counts	Index of relative abundance based on the number of encounters with species per weighted block of time. Plotting a species discovery curve	Crude comparison of abundance between species within a site and within species between sites. Data collection is fairly simple, allowing the observer freedom to roam	Underestimation of flocking species	
Mist-netting	Secret understorey species, index of relative abundance when use of standardized net length and time	Detect understorey birds, get to know the birds	Proper training and special equipment required, time-consuming, mostly limited to understorey species, not cost-effective, capture conditions introduce strong bias	Expensive

Table 1. Bird survey and count methods (adapted from Bibby *et al.*, 1998).

There are some practical considerations for choosing a certain sampling pattern and distance between sampling units (Robinson *et al.*, 2000). In a mountainous rainforest with dense understorey using existing paths or roads may be the method of choice because: a) observers could get lost otherwise, b) finding random points would be very time consuming and c) to get to these points a lot of vegetation needs to be cut down which is not only time consuming but may also be quite destructive. Furthermore, some canopy species are impossible to detect when obscured by foliage, thus using a road may enable you to see them (MacKinnon & Phillips, 1993). When cutting transects during the breeding season there is a danger to destroy nests and you will open pristine forest to people and animals that may follow your tracks to exploit the forest. The disadvantage is obvious – you will not cover your study area evenly, thereby not encountering some bird species that you may have found using a random approach. In forest, two sampling points should be at least 150 to 200 m apart, in open habitat even further. Doing your survey in open farmland savannah it will be more easy to set up transects or to find random points.

- **Distance sampling** (from Bibby *et al.*, 1998)**.** Quantitative methods often require the estimation of the distance between the observer and the bird (Fig. 4). Errors can be minimized by practising beforehand, and it is important that different team members synchronize their estimates. Optical range finders can be useful when you see a bird (but not when you hear it). If you sample from points you can mark certain distances in advance. However, in dense vegetation neither of these methods will work. Estimation of the distance to a calling bird can be practised by placing a tape recorder at various distances. To make things easier one can use distance bands, *i.e.* within 5, 10, 15, 20, 30, 40, 60, 80, 100 m of the observer.

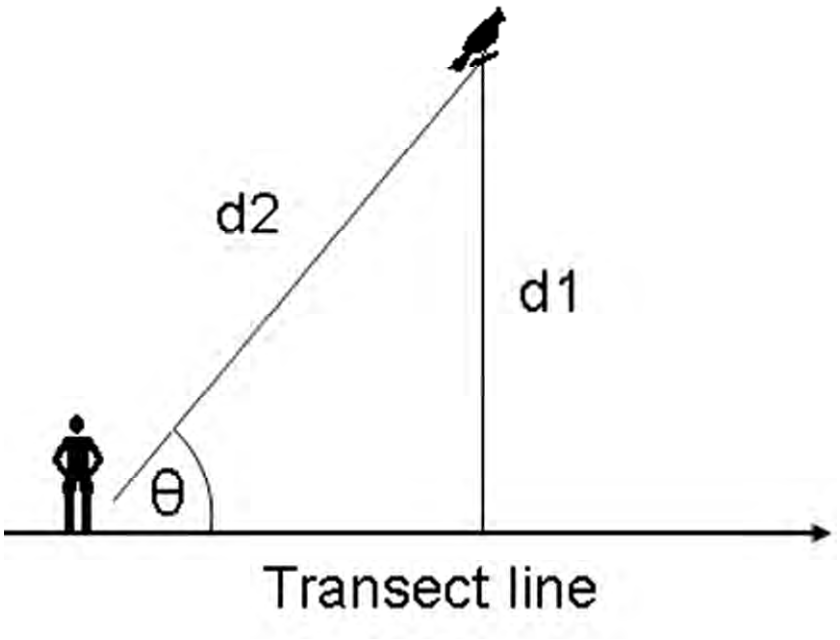


Fig. 4. d1 (perpendicular distance) can be calculated using d2 and the sighting angle θ (d1=d2*sin(θ)).

For the analysis of these data the software "Distance" can be used (Laake *et al.*, 1994). Please check Bibby *et al.* (1998) for further detail. Distance sampling can be used both in line counts and point counts (see Table 2 for advantages of each method).

- **Point Counts.** Points are usually laid out on a random transect, *i.e.* every 50 m. One walks from one point to the other, stops at the point for a predetermined amount of time (*i.e.* 5-10 min) to count all birds present (individuals and species) and then walks to the next point to repeat this and so forth. Distance between points and amount of time spent counting need to be adapted to the habitat. For more details see Bibby *et al.* (1998).
- **Line Counts.** You walk continuously along a certain line and record all bird contacts either side of the track. Walking speed should be constant, a goal very hard to achieve, especially in dense forest. Avoid counts along streams and rivers as your splashing about will flush birds along the river often long before you have even had a glimpse on them. For more details see Bibby *et al.* (1998).

Line Counts	Point Counts
+++ extensive, open and uniform habitats	+++ dense forest or scrub
+++ mobile, large or conspicuous species and those that easily flush	+++ cryptic, shy, skulking species
+++ low population densities or species poor	+++ high population densities or species rich
Cover the ground quickly and efficiently recording many birds	Time is lost whilst walking between points, but at point more time for observation and identification
Double counting minor as observer is on the move	Double counting potential problem
Birds are not so much attracted to observer	Curious birds may be attracted to the observer
+++ when easy access	+++ when difficult access
Can be used for bird-habitat studies	Better suited to bird habitat studies
Errors in distance estimation have a smaller influence on density estimates (because the area sampled increases linearly from the transect line)	Errors in distance estimation can have a larger influence on density estimates (because the area sampled increases geometrically from the sampling point)

Table 2. Differences between point and line counts (modified from Gregory *et al.,* 2004), +++ = good for.

- Territory mapping and other methods. For more advanced methods like territory mapping we refer to the literature (i.e. Bibby *et al.*, 2000). A method especially designed for tropical environments is “Multi Time-Window Transect-Mapping” (see Jahn, in press).

3.4. Mist-netting

Mist-netting is useful to learn the birds in an area and to discover skulking understorey species but it is not time effective and the data obtained are not useful for a quantitative description of the local bird community. According to Remsen & Good (1996), the record of birds through mist-netting measures the activity of birds within 3-4 m above the ground rather than the community composition in the habitat. Mist-netting is very time-consuming, extremely weather dependant and should only be carried out by well-trained people. A good description of how to responsibly use mist nets to catch birds is given in Redfern & Clark (2001). Training is offered in many countries by institutions that organize the national bird ringing schemes.
Europe: http://www.euring.org/national_schemes/contact_schemes.htm

Out Europe:
http://www.euring.org/national_schemes/non_euring_schemes.htm.

Fig. 5. A mist-net of 6 m height using bamboo poles to catch birds in Madagascar. (Photo: F. Woog).

Also see Hofmann *et al.* (this manual) for a detailed description of the use of mist-nets for catching bats. For most passerines, 16 mm Nylon mesh is used, nets are 6 or 12 m long and have 4-5 shelves (giving them a height of about 2.5 m). Mistnets can be placed on ridges, in thickets or at forest edges, where birds often pass close to the ground. If nets are set in wrong places a lot of damage can be done to the birds (bird colonies, roosting places and nests have special rules). Generally, avoid putting the nets in the sun, where they are easily visible, and where captured birds can rapidly dehydrate. Taking birds out of the nets, and handling them, has to be done properly and enough people need to be present to monitor the nets that have been put up at close intervals (every hour, or more often under warm conditions or when it drizzles; but close the nets when it rains!). After extraction from the net birds are usually placed in double-sowed light cotton bags for further processing.

Birds can transmit diseases. To reduce this risk, make sure that bird bags stay dry and clean (turn them inside out and shake them after each capture, and wash them often). Make sure to thoroughly disinfect nets, especially when moving between countries or continents. It is unacceptable to use mist-nets first in an European country and then use them, unwashed, in a pristine tropical forest (the same is of course true for all your camping and outdoor equipment). Also observe principles of hygiene: wash your hands, preferably with disinfective soap. Do not take soiled bird bags into the tent you sleep in.

When you have the bird in your hand, make careful records (photos, weight, fat score, brood patch, moult stage, wing length and other measurements) (Svensson, 1992; Baker, 1993) and, depending on additional research questions, take a blood sample for DNA-analysis or feather for the analyses of stable isotopes (see paragraph on collecting birds). Bird ectoparasites are often poorly known and collecting them may yield new species (preserved in 2 ml vials in 70% alcohol, use very fine tweezers).

Canopy nets can increase the number of species you catch. They are not easy to handle. The best way to learn about these is to ask somebody that uses them.

To quantify birds, mist-netting is not really a good method and will only be useful to compare relative abundances of selected understorey species (see Table 1). If this is planned, the birds need to be marked to avoid double counting recaptures as new captures. The most useful are bird rings, but if these are not available tail feathers can be clipped systematically to identify birds which have already been caught. Clippings should be as small as possible. As birds moult their feathers at least annually, these markings remain temporary. Mark-recapture methods are useful for a wide variety of studies and purposes and can help to estimate abundance of selected species quite precisely or establish local movement pattern (for information about data analysis of capture-recapture data see http://warnercnr.colostate.edu/~gwhite/mark/mark.htm).

A note of caution – locals often ask for mist-nets (as a means of catching birds for food, plumage or for the pet-trade). Never leave mist-nets unattended. When storing them make sure they are as safe as your money and passport. On the other hand, some locals may be afraid of the nets and will not pass by. Make sure you talk to the village people that may encounter the nets, ask them for their permission and explain what you are doing. In some areas with large game, goats, cows or monkeys it may not be advisable to use mist-nets as these animals can easily destroy your expensive nets and may severely injure themselves during the process of entanglement.

3.5. Collecting birds

For a bird survey and monitoring scheme the collection of birds is usually not necessary, but sometimes new species remain undiscovered because birds were not collected. There are all sorts of opinions about collecting birds (see Remsen, 1995; Collar, 2000). Habitat loss, agricultural practice and world-wide climate warming are the real threats to birds, and in comparison the “sacrificing” of a few birds for science means nothing for most species except when they are very rare. But all collecting should be done legally and justified by some clear purpose, such as needs for documenting new-discovered populations and potential new taxa. In many developing countries there exists a sort of split moral – one for indigenous people, one for industrial enterprises and one for (foreign) biologists. Whereas indigenous people hunt birds for a living, and international companies destroy vast pristine natural areas (*i.e.* for mining, dam projects etc.) often, especially foreign, biologists are not being granted collection and export permits for birds. Even the collection of feathers and birds found

dead along roadsides may not be permitted and the export from the country of origin and import into your home country is not easy (*i.e.* concerning species listed under CITES or under health regulations).

However, it is always useful to prepare birds found dead or for a specific scientific question, and local partners can advise on what to do concerning permits. The easiest is usually a cooperation with a museum or university where specimens can be kept before permits have been worked out. It is fair enough that these institutions often want a share of the collected material for their own collections. For preparation techniques see Wagstaffe & Fidler (1968), Harrison & Cowle (1970), Piechocki (1998), Winker (2000) and Hofmann *et al.* (this volume). To see a video on bird preparation, paste mms://137.229.54.15/bts/birdprep.wmv into your browser.

In order to preserve bird skins under field conditions it is best to prepare the skin right away, and to quickly dry it properly (*i.e.* using a kerosene lamp for heating, when it rains). Remove the brain and as much tissue as possible. Salt can be used as a cheap and easily available preservative agent. Thymol can help to prevent bacterial growth and moulding, *i.e.* put some crystals into your air-tight storage containers, and if needed silica gel. If one has no time to make skins in the field or is working in climates with a high humidity, birds can be put into 70 % alcohol (1/3 animal, 2/3 alcohol). When preserving a complete animal without skinning it you need to inject alcohol with a syringe into the internal cavity of the animal and the brain (through the nose). Because the alcohol gets diluted by the fluids of the animal, it is advisable to change it after a few days. Alcohol can wash out certain colours, which is a disadvantage. For transport, the alcohol can be drained and specimens be put into double Ziplock bags. It is also useful to collect a tissue sample (*i.e.* muscle or liver) in the field. This is to be stored in pure 90-95% alcohol or EDTA-buffer. For good practices in tissue conservation: http://www.mip.berkeley.edu/mvz/collections/opportunistic_collection_of_tissue.pdf.

For many research purposes it may suffice to take a blood or feather sample and then releasing the bird (note however, that official permits may still be needed!). Blood samples are useful for genetic studies on various levels not only for speciation but also for population differences (Gaunt, 1999; Dawson, 2005). Stable isotopes found in feathers can give you an indication where a migratory bird grew a feather (Bearhop *et al.*, 2000; Wassenaar & Hobson, 2001). A small drop of blood is taken from the wing vein or in species with soft legs (like swans, geese and ducks) or young birds from the leg vein and placed in small vials containing buffer (200-300 µl blood in storage buffer containing 10% EDTA, 1% SDS, 0.5% NaF, 0.5% thymol and 100 mM Tris, pH 7.4 (Wink, 2006) or alternatively DMSO-buffer (SSDE) consisting of 20% DMSO, 0.25M EDTA pH 8.0 saturated with NaCl). These samples can be stored at ambient temperatures, but longer-term storage at -20°C (or lower) is recommended. Care should be taken, that syringes or buffer do not contain heparin, as this will inhibit the PCR reactions. The procedure should be learned from another ornithologist that has used the method before. Veterinarians often do not have experience extracting blood from birds.

4. Documentation

4.1. Labels

When collecting samples make sure they are properly labelled, a collection number is not enough. A proper label should at minimum contain date (write out the month *i.e.* 11 Dec 2009, not 11.12.2009 as this may be read 11/12/2009 which could be interpreted as 12 Nov 2009, always write the complete year as 09 could mean 1909, 1809 or 2009), exact location including country, species, collector, collection number. Never trust that you will remember to do this later.

http://olla.berkeley.edu/ornisnet/?q=node/5 gives detailed tools and guidelines for geo-referencing. If possible, note longitude and latitude (*i.e.* read from your GPS or map).

4.2. Proper documentation

Field notes should always be detailed, with date, time of day, weather observation (that may influence your survey results, *e.g.* heavy rainfall) and, if possible, number of individuals encountered, and, if discernible, their sex and age. If you see a group of peacocks, for example, note number of males, females, immatures and juveniles. If you encounter birds that you do not know, try to take a photograph, take a sound recording or immediately make a small sketch of what it looked like. If you don't have enough time in the field or you can't write things down whilst you are observing birds, MP3 players can serve as dictaphone. However, always think about the time it will take to transcribe the information from your recordings. One can only guess how many recordings have been made in ornithological research without ever having been analyzed. Modern digital cameras offer a unique possibility for improving field identification. For instance, when a mixed feeding party of birds passes through the vegetation, take as many photographs as you can, and by zooming in afterwards you can identify birds that you did not immediately have time to identify (or later blow up the images on your computer screen). In this way you may sometimes be able to reliably identify every bird in the party.

It is often useful to enter data in forms prepared in advance, as this may facilitate later data entry into your database. The forms should mirror the structure of the database you intend to use. Enter your data as quickly as possible.

When working in wet climates, working with normal paper is a challenge as at the end of your expedition you may end up with a heap of 'papier mâché'. Fortunately there are solutions, *i.e.* "Write in the Rain" notebooks and copying paper (Darling Corporation, http://www.riteintherain.com). These items are not cheap but are well worth the investment. If you use pencil you can drop them in a river and will still be able to read what you have written.

4.3. Database and data analysis

This should be in place before you start the survey, as they are very much linked. Good guidance can be found in Bibby *et al.* (1998, 2000) and Voříšek *et al.* (2008). The statistical approaches for estimating bird abundance from bird counts and taking detectability into account (*i.e.* Kéry, 2008) are well beyond the scope of this chapter. Whenever in doubt, consult a professional ornithologist or statistician before you start fieldwork.

5. Case studies

5.1. Case study 1 from a tropical cloud forest (The Chelemhá, Guatemala)

Combined line-point counts are often used in the tropics. Almost any method is biased to sample the entire bird community (Terborgh *et al.*, 1990; Poulsen, 1994; Remsen, 1994; Remsen & Good, 1996). Therefore, a combination of several methods is sometimes essential to get a complete species list and estimate relative abundance. In Guatemala, it proved essential to combine point counts with transects, since a large part of the bird community would have been missed if using only one method (Renner, 2003). To circumvent losing some essential species, point counts were combined with transects counts: point count sites were established each 25 m along transects. At each 25 m mark, all birds sighted or heard within a nominal distance of 100 m were recorded for five minutes. After the five minutes, the distance to the next point count locality was slowly followed in the shortest possible way covering the distance in about the same time. Transects were 150 m apart totalling 3,300 m. The local cloud forests, the major habitat in Chelemhá, fortunately do not have a very dense understorey, hence only minor efforts were needed to establish the point-transects. The bordering secondary vegetation, however, was very dense, and establishing trails to count birds was impossible (the dense secondary vegetation was a wall consisting of 2 cm thick stems of plants only 20 cm apart at the time of monitoring). The Guatemalan authorities and the land owner were interested in the results of the bird survey but because the area was a non-use forest reserve, all cutting of vegetation was banned. The establishment of point sites and transects was therefore a trade-off between scientific desire (random) and conservation (using existing trails as much as possible and minimizing the impact on the area). To diminish effects of detectability (Hines, 2006; MacKenzie *et al.*, 2002, 2003), all point counts/transects were visited three times a year. The data were used to establish relative abundance of all bird species and to determine presence of species (Magurran, 1988; Rosenzweig, 1995). Results showed that while more species were present in secondary forest, all species of conservation concern were only present in natural forest (Renner, 2003, 2005).

5.2. Case study 2 from cloud forests in the mountains of the tropical Andes

Because of the steepness of the terrain, impenetrable vegetation and lack of trails it was difficult to standardise the sampling and to obtain reliable bird density data (Bibby *et al.*, 2000). Rather than trying to get absolute quantitative data from one or two study plots, the study aimed to obtain semi-quantitative data for comparing the community composition of samples over several sites and habitats.

Avian community data were obtained during transect walks. All visual and acoustical records of birds within 50 m (Schieck, 1997) were noted while walking very slowly and quietly through the terrain and as "randomly" (with frequent changes in direction) as topography and vegetation permitted (Fjeldså, 1999; Herzog *et al.*, 2002). Species accumulation curves level out rapidly (much more so than with point sampling!) and high correlations between relative species abundance data obtained this way and by point-counts in the same area suggests that observations made during "random" walks are not significantly more biased than those obtained by more standardised point counts (Fjeldså, 1999). The main advantages of this "random-walk" approach are the broad sampling of the study area, time-efficiency (all bird observations being used, unlike in point counts; see below) and relative observer independence (Sauer *et al.*, 1994) compared to timed-species-count methods.

At each study site, data were collected within 1-1½ km² and over 2-4 days. Variation in the extent of study plots is not of a magnitude that requires adjustment for area differences. Study sites of this size will represent habitat mosaics (of different associations of forest trees, tree-fall gaps, landslides and glades) but the study plot was large enough to find most birds on the move, singly or in mixed feeding parties. Walking speed varied (as the vegetation is sometimes nearly impenetrable) but was usually ca. 500 m per hour. On average, 0.4 birds were identified per minute. This rate could be raised by walking faster, but the data will then be more biased towards easily detectable species.

The observed species richness is constrained by sample size, and for comparison it is necessary to estimate species richness by extrapolation. Such estimators reach their own asymptote much sooner than sample-based rarefaction curves, they level off and approximate empirical asymptotes well.

The simplest approach (which can be applied currently, during field work) is to use the Chao 1 formula (Colwell & Coddington, 1994):
$S1 = Sobs + a2/2b$ (*Sobs* being the number of species recorded, *a* being the number of singletons = number of species recorded only once, *b* the number of doubletons).

A more sophisticated estimation can be done later using Colwell's software EstimateS (http://viceroy.eeb.uconn.edu/estimates). Ranked abundance curves can be constructed from the total list of observed birds along the route, assuming that the attentive observer is able to detect all birds (at least those which are active) within 50 m from a transect. In most cases, about 500 bird

identifications will provide a good sample for describing the bird community within a study plot.

John MacKinnon and Karen Phillips on birdwatching in forest:*…" Watching birds in tall forests is not easy. You may walk for an hour without seeing anything then suddenly be surrounded by so many twittering birds that you cannot focus on any. A bird may be so high up and so obscured by foliage that you cannot get a good view. In the rain, water on your lenses may blur your vision… leeches are an accepted irritation"…*

6. Acknowledgements

We do sincerely thank Michel Louette (Royal Museum for Central Africa, Tervuren, Belgium), Dieter Oschadleus (Animal Demography Unit, University of Cape Town, South Africa), Olaf Jahn (Aves & Conservación, Corporación Ornitológica del Ecuador*),* Richard Gregory (RSPB, England, EBCC), Andy Musgrove and David Noble (BTO, Thetford, England) and Hans-Günther Bauer (Max-Planck Institute for Ornithology and EBCC, Radolfzell, Germany) for helpful discussions, ideas and comments on earlier drafts of this manuscript.

7. References

7.1. Reference List

AMERICAN ACADEMY OF ORTHOPAEDIC SURGEONS (AAOS) 2008. *Wilderness First Aid*. Jones and Bartlett Publishers. Boston, Toronto, London, Singapore: 369 pp.

BAKER, K. 1993. *Identification Guide to European Non-Passerines*. BTO Guide 24. Thetford: 332 pp.

BEARHOP, S., PHILLIPS, R.W., THOMPSON, D.R., WALDRON, S. & FURNESS, R.W. 2000. Variability in mercury concentrations of Great Skuas Catharacta skua: the influence of colony, diet and trophic status inferred from stable isotope signatures. *Marine Ecology Progress Series* 195: 261-268.

BIBBY, C., JONES, M. & MARSDEN, S. 1998. *Expedition Field techniques: Bird Surveys*. Royal Geographical Society, London. [Online], Available: http://biology.kenyon.edu/courses/biol229/fieldmanual%20birds.pdf [2009, Dec].

BIBBY, C.J., BURGESS, N.D., HILL, D.A. & MUSTOE, S.H. 2000. *Bird Census Techniques*, 2nd edition, Academic Press, London: 277pp.

BIRDLIFE INTERNATIONAL 2000. *Threatened Birds of the world*. Lynx Edicions and Birdlife International, Barcelona and Cambridge 852 pp.

BORROW, N. & DEMEY, R. 2004. *Birds of Western Africa*. Helm Field Guides, London: 551 pp.

COLLAR, N. 2000. Opinion. Collecting and conservation: cause and effect. *Bird Conservation International* 10(1): 1-15.

COLWELL, R.K. & CODDINGTON, J.A. 1994. Estimating terrestrial biodiversity through extrapolation. *Philosophical Transactions of the Royal Society (Series B)* 345: 101-118.

DANIELSEN, F., BURGESS, N.D. & BALMFORD, A. 2005. Monitoring matters: examining the potential of locally-based approaches. *Biodiversity and Conservation* 14: 2507-2542.

DANIELSEN, F., BURGESS, N.D., BALMFORD, A., FJELDSÅ, J., ANDRIANANDRASANA, H.T., BECKER, C.D., BENNUM, L., BRASHARES, J.S., CHRISTIANSEN, S., DONALD, P.G., EGUINO, S., ENGHOFF, M., FUNDER, M., GRAY, M., HÜBERTZ, H., JONES, J.P.G., OETTING, I., POULSEN, M.K., RIJSOORT, J., STUART-HILL, G., TOPP-JØRGENSEN, E., TOWNSEND, W.R., UYCHIAOCO, A.J., WHITTEN, T. & YONTEN, D. 2006. Monitoring matters: evaluating locally-based biodiversity monitoring in developing countries. *Oryx* 40: 14-15.

DANIELSEN, F., MENDOZA, M.M., TAGTAG, A., ALVIOLA, P.A., BALETE, D.S., JENSEN, A.E., ENGHOFF, M. & POULSEN, M.K. 2007. Increasing conservation management action by involving local people in natural resource monitoring. *Ambio* 36: 566-570.

DAWSON, A. 2005. Techniques in physiology and genetics. *In* : Sutherland, W.J., Newton, I. & Green R.E. (Eds). *Bird Ecology and Conservation. A Handbook of Techniques*. Oxford University Press, Oxford: 211-231.

FJELDSÅ, J. 1999. The impact of human forest disturbance on the endemic avifauna of the Udzungwa mountains, Tanzania. *Bird Conservation International* 9: 47-62.

GAUNT, A.S., ORING, L.W., ABLE, K.P., ANDERSON, D.W., BAPTISTA, L.F., BARLOW, J.C. & WINGFIELD, J.C. 1999. *Guidelines to the use of wild birds in research*. The Ornithological Council, Washington DC: 56 pp.

GIBBONS, D.W. & GREGORY, R.D. 2006. Birds. *In* : Sutherland W.J. (Ed.). *Ecological Census Techniques*, 2nd Edition. Cambridge University Press, Cambridge: 308-344.

GILBERT, G., GIBBONS, D.W. & EVANS, J. 1998. *Bird Monitoring Methods: a manual of techniques for key UK species*. RSPB, Sandy: 464 pp.

GREGORY R.D, GIBBONS, D.W & DONALD, P.F. 2004. 'Bird census and survey techniques'. *In* : Sutherland, W.J., Newton, I. & Green R.E. (Eds). *Bird Ecology and Conservation. A Handbook of Techniques*. Oxford University Press, Oxford: 17-55.

HARRISON, C.J. & COWLES, G.S. 1970. *Birds: Instructions for Collectors*. 1st edition. British Museum, London: 48 pp.

HERZOG, S.K, KESSLER, M. & CAHILL, T.M. 2002. Estimating species richness of tropical bird communities from rapid assessment data. *The Auk* 119: 749-769.

HINES, J.E. 2006. *PRESENCE2- Software to estimate patch occupancy and related parameters*. United States Geological Survey–Patuxent Wildlife Research Center (USGS-PWRC). [Online], Available: http://www.mbr-pwrc.usgs.gov/software/presence.html [2009, Dec].

JAHN, O. in press. Bird communities of the Ecuadorian Chocó: a case study in conservation. *Bonner Zoologische Monographien* 56.

KÉRY, M. 2008. Estimating abundance from bird counts: Binomial Mixture Models uncover complex covariate relationships. *The Auk* 125(2): 336-345.

LAAKE, J.L., BUCKLAND, S.T., ANDERSON, D.R. & BURNHAM, K.P. 1994. *Distance User's guide Version 2.1*. Colorado Cooperative Fish and Wildlife Research Unit, Colorado State University, Fort Collins: 84 pp.

LOWEN, J.C., BARTRINA, L., CLAY, R. & TOBIAS, J. 1996. *Biological surveys and conservation priorities in eastern Paraguay*. CSB Conservation Publications, Cambridge: 186 pp.

MACKENZIE, D.I., NICHOLS, J.D., LACHMAN, G.B., DROEGE, S., ROYLE, J.A. & LANGTIMM, C.A. 2002. Estimating site occupancy rates when detection probabilities are less than one. *Ecology* 83: 2248-2255.

MACKENZIE, D.I., NICHOLS, J.D., HINES, J.E., KNUTSON, M.G. & FRANKLIN, A.B. 2003. Estimating site occupancy, colonization and local extinction when a species is detected imperfectly. *Ecology* 84: 2200-2207.

MACKINNON, J. & PHILLIPS, K. 1993. *A Field Guide to the Birds of Sumatra, Java and Bali*. Oxford University Press, Oxford: 491 pp.

MAGURRAN, A.E. 1988. *Ecological diversity and its measurement*. Princeton University Press, Princeton: 179 pp.

MERRY, W. 1994. *The Official Wilderness First Aid Guide*. McClelland & Stewart, Toronto: 408 pp.

NG'WENO, F. 2008. Pitfalls in ethno-ornithological research. *In* : Harebottle D.M., Craig A.J.F.K., Anderson M.D., Rakotomanana H. & Muchai M. (Eds). *Proceedings of the 12th Pan-African Ornithological Congress.* Animal Demography Unit, Cape Town: 44-45.

PIECHOCKI, R. & ALTNER, H.-J. 1998. *Makroskopische Präparationstechnik. Teil I, Wirbeltiere*. Verlag Gustav Fischer, Jena, Stuttgart, Lübeck, Ulm: 461 pp.

POMEROY, D. & TENGECHO, B. 1986. Studies of birds in a semi-arid area of Kenya. III – The use of "timed species-counts" for studying regional avifaunas. *Journal of Tropical Ecology* 2: 231-247.

POULSEN, B.O. 1994. Mist-netting as a census method for determining species richness and abundances in an Andean cloud forest bird community. *Le Gerfaut* 84: 39-49.

POULSEN, B.O. 1996. Structure, dynamics and home-range of two mixed species flocks and species composition in a montane Alder dominated secondary forest in Ecuador. *Journal of Avian Biology* 12: 333-343.

POULSEN, B.O., KRABBE, N., FRØLANDER, A., HINOJOSA, M.B., & QUIROGA, C.I. 1997. A rapid assessment of Bolivian and Ecuadorian montane avifaunas using 20-species lists: efficiency, biases and data gathered. *Bird Conservation International* 7: 53-67.

PRESTON, F.W. 1948. The commoness and rarity of species. *Ecology* 29(3): 254-283.

RASMUSSEN, P.C. & ANDERTON, J.C. 2005. *Birds of South Asia. The Ripley Guide*. Vols. 1 and 2. Smithsonian Institution and Lynx Edicions, Washington, D.C. and Barcelona: 378 & 685 pp.

REDFERN, P.F. & CLARK, J.A. 2007. *Ringer's manual*. BTO, Thetford: 270 pp.

REMSEN, J.V. 1994. Use and misuse of bird lists in community ecology and conservation. *The Auk* 111: 225-227.

REMSEN, J.V. 1995. The importance of continued collecting of bird specimens to ornithology and conservation. *Bird Conservation International* 5: 145-180.

REMSEN, J.V. & GOOD, D.A. 1996. Misuse of data from mist-net captures to assess relative abundance in bird populations. *The Auk* 113: 381-398.

RENNER, S.C. 2005. The Resplendent Quetzal (*Pharomachrus mocinno*) in the Sierra Yalijux, Alta Verapaz, Guatemala. *Journal of Ornithology* 146:79-84.

RENNER, S.C. 2003. *Structure and diversity of cloud forest bird communities in Alta Verapaz, Guatemala, and implications for conservation*. Niedersächsische Staats- und Universitätsbibliothek Göttingen. [Online], Available: http://webdoc.sub.gwdg.de/diss/2003/renner/renner.pdf [2009, Dec].

ROBERTS, D.L., COOPER, R.J. & PETIT, L.J. 2000. Flock characteristics of ant-following birds in premontane moist forest and coffee agroecosystems. *Ecological Applications* 10(5): 1414-1425.

ROBERTSON, H. & HEATHER, B. 2004. *The Hand Guide to the Birds of New Zealand*. Oxford University Press, London: 168 pp.

ROBINSON, W.D., BRAWN, J.D. & ROBINSON, S.K. 2000. Forest bird community structure in central Panama: influence of spatial scale and biogeography. *Ecological Monographs* 70: 209-235.

ROSENZWEIG, M.L. 1995. *Species diversity in space and time*. Cambridge University Press, Cambridge: 436 pp.

SAUER, J.R., PETERJOHN, B.G. & LINK, W.A. 1994. Observer differences in the North American Breeding Bird Survey. *The Auk* 111: 50-62.

SCHIECK, J. 1997. Biased detection of bird vocalizations affects comparisons of bird abundance among forested habitats. *Condor* 99: 179-190.

SVENSSON, L. 1992. *Identification Guide to European Passerines*. 4th edition, revised and enlarged, self-published, Stockholm: 367 pp.

TERBORGH, J., ROBINSON, S.K., PARKER, T.A., MUNN, C. & PIERPONT, N. 1990. Structure and organization of an Amazon forest bird community. *Ecological Monographs* 60: 213-238.

VALLELY, A.C. 2001. Foraging at army ant swarms by fifty bird species in the highlands of Costa Rica. *Ornitologia Neotropical* 12: 271–275.

VOŘÍŠEK, P., KLVAŇOVÁ, A., WOTTON, S. & GREGORY, R.D. 2008. *A Best Practice Guide For Wild Bird Monitoring Schemes*. Royal Society for the protection of Birds, Sandy: 150 pp.

WAGSTAFFE, R. & FIDLER, J.H. 1968. *Preservation of Natural History Specimens*. Littlehampton Book Services Ltd., vol.2, part 2: Zoology: Vertebrates. Witherby, Philosophical Library of London: 115 pp.

.

WASSENAAR, L.I. & HOBSON, K.A. 2001. A stable-isotope approach to delineate catchment areas of avian migration monitoring stations in North America. *Environmental Science and Technology* 35: 1845-1850.

WERNER, D. 1979. *Where there is no doctor*. *A village health care handbook for Africa*. McMillan, Oxford: 440 pp.

WINK, M. 2006. Use of DNA markers to study bird migration. *Journal of Ornithology* 147: 234-44.

WINKER, K. 2000. Obtaining, preserving, and preparing birds. *Journal of Field Ornithology* 71: 250-297.

7.2. Useful Internet links

Birdlife International.
http://www.birdlife.org/regional/index.html

Sound archives.
http://www.bl.uk/soundarchive
http://www.birds.cornell.edu
http://www.xeno-canto.org/africa/index_static.html
http://www.xenocanto.org/index_static.html
http://www.xeno-canto.org/asia/index_static.html
http://www.xenocanto.org/australasia/index_static.html

Bird collections.
http://www.museum.lsu.edu/~Remsen/AVECOLlections.html
http://www.scricciolo.com/European_Bird_Collections_C%20S%20Roselaar.pdf
http://olla.berkeley.edu/ornisnet/ (American Bird collections, ORNIS)

Bird species.
http://avibase.bsc-eoc.org/avibase.jsp

Online access to bird collection data.
http://www.gbif.org, http://neotropical.birds.cornell.edu/portal/home
http://www.zmuc.dk/VerWeb/Tanzanian_Vertebrates/TanzVert.index.html

Bird monitoring.
http://monitoringmatters.org

South-African Bird Atlas.
http://sabap2.adu.org.za/index.php

Guidelines to the use of wild birds in research.

http://www.nmnh.si.edu/BIRDNET/GuideToUse/Guidelines_2d_edition.pdf

European Bird census council.
http://www.ebcc.info/index.php?ID=365

Waterproof paper.
http://www.riteintherain.com (Darling Corporation)

Tissue collection.
http://www.mip.berkeley.edu/mvz/collections/opportunistic_collection_of_tissue.pdf

Bird preparation.
http://www.uaf.edu/museum/bird/personnel/KWinker/Winker%20specimen%20preparation%20J%20Field%20Ornithol%202000.pdf
http://www.springerlink.com/content/100125/

Data analysis of capture-recapture data.
http://warnercnr.colostate.edu/~gwhite/mark/mark.htm

7.3. Books

Voříšek *et al.*, 2008. A Best Practice Guide for Wild Bird Monitoring Schemes. http://www.ebcc.info/index.php?ID=365

Sutherland *et al.*, 2004. Ecological Census Techniques, see section on “Gratis Book Scheme”.
http://assets.cambridge.org/97805218/44628/frontmatter/9780521844628_frontmatter.

Chapter 22

Preservation of freshwater fishes in the field

by

Dirk Neumann
Zoologische Staatssammlung München
Section Ichthyology / DNA-Laboratory
Münchhausenstr. 21, 81247 Munich, Germany
Email: Dirk.Neumann@zsm.mwn.de

Abstract

Collecting in rivers, streams or lakes is challenging, and the fishing efficiency is highly depending on habitat conditions and the selection of fishing gear. While some fishing gear can be very targeted, most gear types result in the capture of non-specific by-catch. Therefore, careful and thorough planning of any field project is essential in ensuring the collection of undamaged, well preserved samples, including the targeted species. Planning not only includes preparatory work before heading into the field such as applying for permits and the selection of suitable fishing gear for the specific habitat and species to be sampled, but also putting together comprehensive sampling equipment for the activities to be undertaken and assembling adequate personnel to handle all sampling events during the specified time period.

Key words: Fishing gear, fishing methods, fishes, preservation, tissue sampling

1. Introduction

“If field workers understand how specimens are processed and used in museums, they will prepare better specimens. If collection users understand how animals are collected and preserved in the field, they will make better use of the specimens. If all of us understand how collections are managed, specimens will be better utilised and preserved for the future.” (Simmons, 2002). Besides your own research interests, specimens in natural history collections serve as valuable representatives of natural populations for other scientists for decades or even centuries. Therefore, all collections from natural (fish) populations should be made careful and with the best preservation procedures to ensure the highest quality of the preserved specimens and tissues for future research. Collection of ancillary material (additional specimens, species or amount of tissue) should be evaluated against the time, effort and money invested in the sampling. With collecting becoming increasingly difficult both due to budget and permitting issues, collaborative collecting is becoming more prevalent and cost effective.

2. Permits, regulations and responsibilities

Ecologists and Biologists working with freshwater fishes have to cope with many regulations and obligations. Besides national and international regulations and provisions on species covered under the *Convention on International Trade in Endangered Species of Wild Fauna and Flora* (CITES), additional permits and licences are necessary or required for endangered, threatened or protected species and have to be considered in the planning of a proposed field work project. These should not only include the required permitting legislation, but also consider the ethical treatment of the collected specimens. Minimizing actions and conditions that might induce physiological stress, physical damage and injury are not only a matter of animal welfare, but may have a direct impact on the quality of the preserved fishes in the field.

2.1. Collecting permits

Prior to the start of any field programme and depending on which country the field work is to be conducted in, all necessary permits for the proposed fishing and sampling activities in the study area must be obtained. This includes official research permits on multiple national/federal levels, fishing permissions, a valid fishing licence at least for the fishing person (mandatory in Europe and North America) and especially local permits (*i.e.* allowance/authorisation of local fishing right holders, communities, village chiefs, etc.). For entry and collecting in several National Parks additional permits might be necessary, negotiated and permitted by the administration of the National Park itself. Official and local permits should include the name of each field crew member on the permit, and the explicit permissions for entry of special geographic locations such as National Parks, restricted/prohibited areas, private land etc., and allowance of

collection of specific species (especially CITES, threatened, endangered or protected species) including any potential by-catch or the collection of other disciplines if applicable. Applications for these official and local permits should be made well in advance of the planned field work with adequate time for processing (and return) of the permits. Illegal fishing without permission may result in fines and other penalties (including prison time in some countries). Any permit conditions should be strictly adhered to (limitation of number of specimens of individual species, return of unwanted material to the environment, return of collected material to the country of origin after the collecting trip, restrictions on methods of collection, etc.).

2.2. Export / Import Permits

An export permit is required if the sampling/field work is carried out in a foreign country and the samples have to be transported from this country or state for further study and analysis (and/or final deposition). If the material is deemed for final deposition at the destination institution, the export permit should specifically allow the permanent export from the host country and transfer of ownership. The transfer of ownership may be negated by conditions such as export of CITES, endangered or protected species, or by other stipulations regarding the return of identified material to the host country. Again, as with collecting permits, any conditions or stipulations of the export permit must be adhered to. Import permits are required for the import of CITES species, but may also be required for the destination country and should also stipulate permanent deposition at the destination institution.

If the collected material is to be transported across federal/state, provincial or county boundaries, or exported and imported to different countries (*e.g.* for entry into the EU), national or international animal health and veterinary regulations may also apply requiring certificates for transportation, *i.e.* health or veterinary certificates, as detailed in Section 5 (Trade measures, importation/exportation procedures and health certification) of the *Aquatic Animal Health Code* as provided by the *Office International des Epizooties* (OIE). For further reading follow the OIE-link given below to Internet based information.

2.3. Additional permits and licences

Besides the above, further permits and licences may be required for any proposed fishing activities, including but not limited to:

- Valid electro-fishing licence at least for the collector who operates the dip net/the anode pole.
- Valid skipper's licence for the boat driver and/or coxswain (either a person of the fishing crew or a hired skipper) who knows the particular river very well (invaluable in navigating unknown, unchartered or dangerous waters).
- Diving certification for all persons who will be engaged in SCUBA sampling.
- Additional permission may also be needed for certain collecting methods, *e.g.* the use of rotenone (especially in freshwater environments), spear

guns, gill nets or any other method not routinely allowed by the general public.

2.4. Conservation and species protection regulations

Besides the relevant permits outlined above, the planned fieldwork has to comply with international wildlife regulations and provisions. The conservation status of the target species, and any potential by-catch in the specific collection area, needs to be evaluated before undertaking the collecting trip, to ensure that the necessary permits are in place prior to collection. Some species are protected or regulated by special legislation, such as CITES, the *International Union for Conservation of Nature* (IUCN), the *European Nature Conservation Legislation* or the *Endangered Species Act* (ESA), etc. Additionally, national or federal regulations on endangered species and species protection may apply or demand the obtaining of exemptions for catching certain species during closed seasons as well as undercutting minimum size limits.

2.5. Fish handling procedures and ethical concerns

Fishes are extremely sensitive animals that require fundamentally different handling requirements compared to other vertebrates due to their physiochemical make-up. Unnecessary by-catch together with careless handling and injury to specimens can result in increased mortality rates and must be avoided. Unnecessary (physiological) stress, inadequate handling or manipulation of specimens in the field will result in discoloured, damaged specimens with limited or no scientific value. Unless the fishes are not already dead (*e.g.* gill net fishing), fishes have to be euthanased prior to tissue sampling. An overdose of approved anaesthetic immobilizes the fish and allows more efficient processing and sampling of the catch and reduces potential pain at contact with the fixative.

Appropriate ichthyological anaesthetics include:

- Chlorobutanol (trichloro-2-methyl-2-propanol, CAS-No. 57-15-8); a saturated solution of approximately 1-2 tablespoons per litre will narcotise within seconds and has no known negative degrading effects on the DNA extracted from tissues. Possible issues:

  a) For euthanasia, dip specimens 3-4 times for few seconds into the anaesthetic, but do not leave them for longer periods in the fluid; the adhering narcotic on the gill surface is sufficient for sedation.

  b) Species with thick mucilaginous layers (*e.g.* eels, sturgeons) show increased mucus secretions after Chlorobutanol treatment caused by the high salt concentrations; especially those species should be narcotised by repeatedly short dipping for only few seconds.

  c) Low ambient temperatures and metabolism rates of fishes during autumn/winter demand higher Chlorobutanol concentrations which may lead to vascular gill swellings and subsequent gill haemorrhage due to the high salt concentrations of the anaesthetic.

d) Air breathing fishes are hardly affected and cannot be efficiently narcotised with this method.

e) Chlorobutanol is extremely dangerous if ingested and may cause irritation of skin and eyes (see Appendix for further information and follow link for MSDS provided in section 7, internet based information).

- MS-222 (9-Tricaine Methane Sulfonate, CAS-No. 886-86-2) is the only approved substance (Europe, North America) for anesthesia of fishes; the fine powder can be dissolved in much lower concentrations (10 mg/l, thus avoiding possible negative effects of high salt concentrations of the narcotic; irritant but less irritating as Chlorobutanol (see Appendix for further information and follow link for MSDS provided in section 7, internet based information).
- Clove oil (CAS-No. 8015-98-2) is a natural analgesic, the main ingredient Eugenol is used as narcotic mainly for marine organisms; Eugenol is water insoluble, for usage emulsify 1-5 ml clove oil in alcohol; irritant and hazardous in case of skin contact (see Appendix for further information and follow link for MSDS provided in section 7, internet based information).
- Carbon dioxide can be an effective narcotic and is easily available *e.g.* carbonated bottled water or soda; narcosis can take longer with this method and may cause body contortions and muscle spasms that may affect the quality of the preserved specimens.

After short sedation in above detailed anaesthetics, or after electrofishing, fishes recover in well-oxygenated and ambient temperate water usually within minutes. For recovery, they should be placed in a separate tank or bucket. Only fully recovered specimens should be carefully released back into their environment to prevent injuries, damage or predation while still tranquilised.

2.6. Fishing relevant safety issues

Dangerous situations during fishing can arise within seconds leading to serious injury or fatalities.

Always ensure a firm, stable footing when collecting near or in water – especially when wading in fast flowing streams, in deep water or in case of low visibility of the water. Be especially cautious around slippery surfaces such as exposed, wet rocks, submerged substrates and vegetation or on moveable and uneven surfaces (sand, boulders etc.), and wear appropriate footwear such as rafting or canoeing shoes. Remember that waders can exacerbate such circumstances by causing extra drag and weight, especially when suddenly infiltrated by water. Attempt to strip off waders if in trouble.

When sampling with nets, beware of entanglement and drag caused by the net which can pull you off balance or into deeper water. If in trouble, release one or both ends of the net to maintain your footing and allow the net to drift with the current to minimize the contact surface and to reduce the velocity pressure and drag force of the net. In case of net fishing from a boat, avoid that the propeller

snags on static or grounded nets or catches the net during retrieving it into the boat. Human life is at all times more valuable than any sampling equipment!

When electrofishing, special precaution should be taken against electrocution by using rubber boots and gloves. Make sure that only certified/trained persons are allowed to operate the electrofishing device (mandatory in several countries). In the event of a member of the team falling into the water or otherwise coming into contact with electric current, cut immediately the electric circuit by removing either the anode pole or cathode rod from the water. Stop the engine before trying to help imperilled persons! Negligence of these simple principles will endanger further lives.

When diving or snorkelling, ensure that all safety precautions are taken and that one member of the team is always on shore to assist in an emergency.

A mobile or satellite phone and GPS device should always be part of sampling safety/emergency equipment to allow for rapid contact of and position location by emergency crews in the event of an accident.

2.7. Fluid fixation/preservation hazards

Exposure to aqueous solutions or fumes of formaldehyde should be avoided by always working in well-ventilated areas or fresh air and through the use of approved protective equipment. Formaldehyde is not only noxious but is also a known carcinogen. Minimise direct exposure as best as possible (*e.g.* only open the fixation container to add further specimens). Latex gloves provide no protection against formaldehyde, use Nitrile or Neoprene gloves for protection. Do not wear soft contact lenses which absorb formaldehyde vapours and will trap them against the eyes (Cohen *et al.*, 1979, cited in Simmons, 2002).

3. Fishing strategies (fishing gear, fishing methods)

Most fishing methods applied for ichthyological sampling catch unspecific, thus it is difficult (or impossible) to target individual species without some element of by-catch. This by-catch can however be reduced through the employment of appropriate, habitat specific sampling techniques, while the collection of multiple species may necessitate the use of multiple techniques at a given site. Fishing efficiency is affected by multiple factors (specifications and selectivity of the gear, seasonal variation, habitat conditions, fish size, etc.) which, in return, directly influence the collection efficiency of the field project. The fishing gear has to be selected in terms of operational efficiency and the availability of target species with regards to the sampling location. “Target species” is used here as a technical term and may include single species confined to specific habitats (*e.g.* specific pelagic fish species confined to open-water habitats which must be targeted with specific net gear), but can also include the complete fish fauna from a specific collection site (*e.g.* for taxonomical collections of fish faunas from previously unexplored rivers or lakes). Depending on what is defined as “target species” for the specific field trip or collection event, the fishing gear has to be selected. During the survey, it might be necessary to adjust the fishing methods to provide the required quality and quantity of the catch. A variety of

gear types or repeated fishing at the same spot might be necessary to ensure the widest possible range of fish species and life stages. Spawning and migratory behaviour of the target species, habitat preferences of different life stages of the same species and a basic knowledge of physical stream parameters, such as velocity, conductivity, stream size, water depth, water temperature, underground conditions (muddy/sandy/rock) are crucial for successfully fishing and sampling. Aspects that influence fishing efficiency are:

Water depth

- Deep water bodies such as lakes or large rivers favours long-line fishing, ground nets, fish weirs (if velocity allows).
- The length of the anode rod limits electrofishing (which normally ends 1 m below the water surface).

Water conductivity

- The salt concentrations in rivers and streams (depending on the geological conditions of the drainage area), at estuaries or entering freshwater streams in euryhaline lakes, in tidal pools and estuaries may vary extremely (*e.g.* favours or limits electrofishing).
- Different salt tolerances/preferences of euryhaline/stenohaline target species.

Water clarity

- Influences snorkelling/SCUBA diving.
- Electrofishing efficiency is strongly influenced by the clarity of the water.

Velocity

- Most static nets can only be deployed in shallow/stagnant water or in the direction of the current at moderate velocity.
- Higher water velocity drastically decreases electrofishing efficiency because stunned fishes will drift faster while increased drag on the anode pole will reduce its manoeuvrability.

Different behaviour/activity of target species

- Pelagic/demersal/benthic species.
- Diurnal/nocturnal species.
- Aestivation/hibernation.
- Spawning migrations.

Remark: Small species sheltering themselves in the shallow water from large nocturnal predators are easily caught at night in the shallow water using headlamps, handnets or a beach seine.

Habitat conditions

- Cobbles, boulders and rocks shelter lithophile or rheophile species.

- Benthic species may hide in muddy or sandy ground.
- Large stones, branches and trunks of trees minimize application of net gear.

Be aware that all of these parameters and conditions can change and normally do change within minutes during heavy rain events, *e.g.* in the rainy season. This not only strongly influences the fishing events following after the rain event because of increased turbidity or cloudiness of the water. Heavy rains (especially overnight) might also necessitate immediate removal of static fishing gear to avoid damage or loss.

Generally speaking, there are two different fishing methods – active and passive. For passive methods (such as gill nets or fish traps), personnel are only required for deployment and retrieving of the gear. Active methods require (with few exceptions) at least two people to actively operate the fishing gear during the collecting period. While active gear can be adapted during fishing *e.g.* for difficult habitat conditions, passive gear cannot. A combination of active and passive fishing methods will raise the sampling efficiency at a collecting site through setting of passive gear before the start of any active method and retrieving thereafter.

3.1. Selective (active) fishing methods

3.1.1. **Cast (throw) net** (Fig. 1A)

Operation

- Small net thrown onto the surface in a circular formation.
- After sinking to the ground the net is closed and retrieved.
- Requires trained skilled person to successfully use a cast net (for further information follow link provided at Internet based information below).

Specifications

- Small mesh sizes (usually 0.8, 1.0, 2.0 cm).
- Net should have bottom pockets to hold the catch.

Application

- Used in streams, rivers, lakes.
- Can be operated either wading in shallow water or from the boat.
- Allows for fairly directed and selective fishing.
- Can be used for retrieving live fish for bait.
- Only applicable at low or moderate water velocities (net will collapse in higher currents before reaching the ground).

Remarks

- The sample site should be free from obstacles like fallen trees, branches, roots, cobbles or boulders to allow closure of the net.
- Repeated casting at the same spot can scare off nearby fishes through the splashing of the net.

3.1.2. Seine (beach/pole seine) (Fig. 1A)

Operation

- Easily deployed from shore, wading in shallow water or from a boat.
- Requires minimal instruction and training.

Specifications

- Large variety of mesh sizes, lead lines and floats are available.
- Net specifications dependant on habitat and size of the target species.
- Should have a bunt (cod end) to effectively trap the catch.

Application

- Effective in most habitats but especially in larger streams, rivers and lakes.
- Shorelines should be free of obstacles to allow net to be pulled onto shore for effective specimen collection.
- Low to moderate water current.
- Beach seine with a fine mesh (up to 1.0 cm) should be shorter (approx. 10 m) to ensure that the seine can be pulled quickly enough against the current.
- Net filament can be either a strong visible (cotton) yarn or a less perceptible nylon or polyester, which provides lower visibility and detection by the catch.
- The lead line must be kept at ground level and ahead of the float line to prevent fish from escaping under the net.
- The float line must be raised well above the water surface when pulling the seine towards the shore to prevent fish from jumping over the net.
- Operating larger beach seines (20 m or more) requires a larger mesh size (to lower the velocity pressure) and more people to pull the net (because of increased drag force).
- Pole seines are usually operated from boats in deep water, or by wading in calm water (*e.g.* lakes).

Remarks

- Water depth and current can affect the efficiency of a seine net.

- If the water conditions allow, nets should be set or operated from row boats (to prevent any propellers from damaging or curling up the net) or manually by multiple people.
- This method has the advantage of being operated quietly, reducing the possibility of scaring fish from the sampling area.

3.1.3. **Frame net** (Fig. 1B)

Operation

- Individual fishing in shallow water.
- Along beaches and under overhanging shoreline vegetation (except for stands of dense weeds).
- For kick-net sampling in shallow riffles.

Specifications

- Either as solid aluminium or metal frame (60 x 40 cm), collapsible (two solid connectable parts) or foldable frame with a solid bar and two movable arms.
- Fine mesh (2.0-4.0 mm) to collect small species and fish fry.
- Approx. 30-40 cm deep net sack.

Application

- In shallow riffles, in creeks, smaller streams, slip-off slopes of larger rivers.
- Frame net is rammed into the soft bottom of the river vegetation and quickly lifted up.
- During sorting of the catch the frame must be kept well above the water surface while the net sack should remain dipped into the water.
- As a kick-net it can be either dragged by the current over larger boulders and rocks or scraped against the current, lifting smaller cobbles and stones and trapping smaller lithophilic fishes.

Remarks

- Favour aluminium frames in areas in which strong-electric fish occur (*e.g.* electric eels in South America or electric catfishes in Africa) due to the amplifying effects of the metal frame.
- Keep the net sack closed when crossing/wading through moderate to strong flowing water to reduce the drag force.

3.1.4. Angling

Operation

- Moderately selective method especially for clear water habitats.
- Species can be targeted through specific baiting.

- Fish size and species depends on hooks (size and form) and bait used.

Specifications

- Different types of fish hooks and monofilament lines, weights, baits, fishing rods and reels.

Application

- Can be employed from shore or boat.
- Highly biased method for fish size and species.
- Most effective if the angler has a specific knowledge of the habitat and habitat preferences of the target species.

3.1.5. **Hand nets** (Fig. 1B)

Operation

- Very selective method which allows also observation of fish activity, behaviour and habitat occupation (while snorkelling or SCUBA sampling).
- For collection of relatively small species in shallow water or from the surface of deeper water.
- Allows specific and selective sampling of individuals (*e.g.* breeding pairs, gobies and their symbiotic shrimps, etc.).
- Can also be used underwater for snorkelling or SCUBA collection.

Specifications

- Commercially available (aquarium) hand nets.
- Fine mesh size on varying size circular or square frame with wooden or metal handle.

Application

- Additional method for collecting specimens that may escape from seine nets.
- For underwater rotenone collections.
- For chasing specimens in very shallow pools and streams where larger seines are rendered ineffective.

3.1.6. Spear (Spear gun, Hawaiian sling or pole spear)

Operation

- Most selective collection method to target individual specimens.

Specifications

- Varying types, sizes, power and spear type (single or multi-barbed).
- Rubber band or air powered spear guns with barbed spear.
- Pole spear – long spear powered by rubber band (Fig. 1D).
- Hawaiian sling – smaller rubber band powered spear.
- Hand spear – thrown into shallow water or at species close to the surface of deeper water.

Application

- In any water body.
- Usually used underwater while snorkelling or SCUBA diving (although restricted or illegal in some countries).
- Requires sufficient underwater visibility.
- Can also be used from the surface on shore or from a boat.
- Hawaiian sling: operated and fired much like a slingshot; rubber bands attached to tube or block through which spear is drawn back and aimed.
- Pole spear: rubber band is held in the hand while pole is drawn back through the hand to produce tension in the rubber band; aimed through extending arm in front of face (Fig. 1D).
- Hand spear: varying length and barbs; effective in collecting flatfish in shallow water.

Remarks

- Spear collection is usually prohibited on SCUBA for the general public and specific permission may be necessary.
- Care should also be taken not to lose the spear and not to fire towards hard substrates or surfaces.
- Caution should be exhibited when using spears to ensure that they are not aimed towards other people or misfired during handling.

3.1.7. Electrofishing

Operation

- Common survey method to collect specimens as well as estimate abundances, density and species composition of fish populations.
- Uses electricity both to stun and attract the fish with the positive pole before capture (Galvanotaxis).
- Needs two persons for operation, one operating the dead man's switch and the electrofishing device, the other for catching and collecting the stunned fish.

Specifications

- Effectiveness depends heavily on water conductivity.
- Influenced by the size of the water body and riverbed conditions (soft or hard ground).
- Various models are available that generate varying strength of electric field.
- Pulsed or non-pulsed direct current (DC).
- Portable battery (Fig. 1B) or gas powered backpack models for small rivers and streams or large stationary models are available which are operated either from shore or a boat.
- The positive pole (anode) usually forms a ring at the end of a 2 m fibreglass or wooden pole and holds fine mesh net.
- The negative pole (cathode) comprises a braided copper cable which trails several meters behind the operator in the water.
- Pulse speed, voltage gradient and current influence and trigger Galvanotaxis and cause fish to turn into the electric field and to be attracted towards anode pole.
- Rubber gloves and rubber boots must be worn to isolate the operator and collector and to prevent electrocution.

Application

- Most useful in small creeks, streams, rivers and shallow (littoral) zones e.g. in lakes, in smaller rapids.
- Additional barrier nets can be placed downstream to collect the catch.
- Operator must be trained and in several countries a valid electrofishing licence is required.

Remarks

- Anode rods with dead man's switch included in the rod should be preferred instead of electrofishing devices with separate dead man switches operated by additional crew members (*e.g.* boat driver) for safety reasons, as the electrofisher operating the anode rod has the best overview in case of emergency.
- Stunned fish must be continuously removed from the electric field and be collected in plastic buckets or containers by additional fishing crew members to avoid injuring the fish through long term muscle contraction or tetanus.
- A continuous tetanus may easily break the backbone of smaller specimens.
- Fishing should be conducted in upstream direction so that disturbed debris and sediment are washed downstream and the visibility of the water remains good.

- Also minimises the escape effect of the cathode and allows for easy collection of the stunned fish that are swept downstream towards the collecting crew.
- The electric field depends not only on the conductivity of the water, but also on the ring size of the anode rod (see Table 1).
- Bedrock and sand bottom are insulating and support a stronger electric field in the free water column while soft or muddy bottoms are weakening and may even cause a collapse of the electric field (this might necessitate shortening the cathode length and thus the surface area with a cable strap).
- Wearing Polaroid glasses increases the visibility of both targeted fish and obstacles under water.
- Using a metal boat as cathode is strictly prohibited in several European countries for safety reasons.
- For further practical information please refer to Section 5.4.1 of the Fish Collection Methods and Standards Manual (website address see below at Internet based Information).

3.1.8. Fish market

To obtain a fast overview for the fish biodiversity especially in large rivers or lakes, it is always valuable to explore and obtain specimens from local fish markets. Local fishermen know the specific fishing grounds in their area well and normally apply a variety of different fishing methods which can provide a surprisingly high species richness. Depending on the climate and geographical region, these fish markets are either stocked early in the morning (before sunrise) or late in the afternoon. It is best to visit fish markets during these times to ensure freshness. Specimens should be sampled immediately on the market or might be carried cooled (*e.g.* placed on ice if available, but might be problematic in remote areas) possible for later sampling to ensure specimen and tissue viability. Fresh fishes are easily recognised by their transparent fins and vividly red coloured gills when lifting the gill cover. In most cases these specimens will need to be purchased and should be purchased to be fixed as voucher specimens, but in some cases either fishermen are not willing to sell their catch (*e.g.* fishermen depending on subsistence fishing in remote villages) or a specimen of a rare species might be too large to fit in any preservation container. In cases like this, ask for allowance of tissue sub-sampling of gill filaments or fins and photograph the specimen, so that the images can be taken to serve as vouchers. Fresh and freshly smoked and dried (not fried) specimens obtained from a fish market are even suitable for DNA sampling. As much authentic collecting information as possible should be obtained from the fishermen (fishing location, habitat conditions, fishing gear, fishing time, etc.), keeping in mind that might be imprecise or wrong, as some fishermen are not willing to reveal exact locations or fishing methods. Be aware that on large fish markets in major cities or capitals valuable species or complete catches might be carried by truck over hundreds of kilometres, stacked in layers of ice and sawdust to be sold for higher revenues. Sawdust in the fish baskets or on the

market place and bent or deformed fish bodies because of being stacked for hours or days are good indicators for this practise.

Commercial fishing methods are omitted here, since they are not applicable for small scale scientific surveys in freshwater environments. To expedite large lakes with trawling equipment, a commercial fishing crew and fish trawler should be hired.

3.2. Unselective (passive) methods

3.2.1. **Gill net** (Fig. 1C)

Operation

- Set across rivers and streams or on lakes (usually at night).
- Left in place for longer periods to allow specimens to become entrapped.
- Sampling areas should be free of underwater obstacles and have a moderate to low current.

Specifications

- Different types of net gear highly specific for species and fish size (standard gill nets, for targeting multiple fish sizes multi-mesh gill nets or enmeshing/trammel nets are used).
- Usually constructed of monofilament so as to be strong and invisible, but also as (visible) multifilament nets.
- Mesh sizes, floaters and weights might be optimised depending on the target species.
- Lead and float line should be sufficient to ensure net remains perpendicular to any current.
- Single nets can be combined to larger panels.

Application

- Highly effective when set in moderate to slow current in rivers, streams and lakes as a single net or as multiple sets in a staggered arrangement parallel to the shore, blocking preferred habitats or shelters (*e.g.* weeds or littoral zones).
- Can be effective for collection of species other nets will not catch (nocturnal, deeper water species).
- Net should be tied off to shore or a heavy weight to ensure net remains in place while floats should be large enough to ensure net does not get dragged under.
- For level inventories nets and panels employed at different depths (*e.g.* for target species with vertical or diurnal/nocturnal migrations).

Remarks

- Monofilament nylon nets should be cleaned after usage from any biofilm or algae coatings to maintain the invisibility.
- Areas with high fish abundances might require repeated control of the nets to minimize the number of killed fishes and possible negative effects on the fish population.
- Reducing the net size (single nets vs. nets combined to large panels) also helps to reduce the catch and avoids over-sampling.
- Might also be used as stop net for electrofishing or invisible seine in small streams with low risk of damages of floating debris (branches, leaves, etc.).
- For further practical information on gill nets and their application please refer to Section 5.3.2 of the Fish Collection Methods and Standards Manual (website address see below at Internet based Information).

Fig. 1 (next page). Active and passive fishing gear. A. Small beach seine (8 m) with lead line and floaters and approx. 1.20 m deep bunt; cast net with bottom pockets, diameter 8.0 m; B. Left, front: battery powered DEKA 3000 electrofishing device (out of production) with anode rod, collapsible frame net (net fixed with Velcro strip to aluminium frame); Middle: collapsible frame net, aluminium frame 60 x 40 cm, mesh size 2.0 mm, net sack 40 cm deep, frame included in special pocket in the net and secured with Velcro strip; Far right: Large dip net (approx. 1.0 x 1.2 m) with lead line and lateral short poles for manoeuvring the net; Front: staple dip nets (10 mm mesh, aluminium frame / 2 mm mesh, stainless steel frames, which might also be used as anode dip net for electrofishing), and hand nets; C. Monofilament gill net with lead line and floats (20 m, 10 mm mesh), fish trap with two traps and connecting wing (approx. 5 m long); Front: foldable fish traps without and with wings; D. Rubber band powered pole spear. (Photos D by M. Geiger, all other photos by D. Neumann).


A
B
C
D

3.2.2. Long-line fishing

Operation

- Short lines (up to 20 m) for sampling along shores or obstructive habitats like rapids or block stone embankments.
- Longer anchored lines (50 m and more) for sampling the river bed of large and deep rivers.
- Multiple hooks on branching lines and baiting may affect sampling efficiency.

Specifications

- Strong braided hook links with high strength fibres for maximum strength and abrasion resistance should be used.
- The length of the hook links should not exceed half the distance of the single hook links to each other to prevent the entangling of the hook lines.
- Small barbed hooks in combination with strong hook links allow catches of small to large species and minimize injuries and improves unhooking.
- Long-lines are usually anchored to the shore or substrate, additional weighting with lead is not necessary.

Application

- Long-line fishing is the only known method to sample the riverbed of large/deep rivers.
- Long lines for river bed sampling must be employed and anchored from a boat and marked with buoys.
- This method is even applicable in strong currents.
- Application of 20 m long-lines from the shore/river bank requires two persons (one holding the baited line which is anchored to the substrate, the other is throwing the anchored free end perpendicular to the beach into the river/stream).
- In strong currents or rapids, the free end fixed to a buoy may be released with the current, the depth of the long-line can be adjusted with length of the buoy line holding the free end.

Remark

- Secure the hooks immediately after unbaiting/unhooking within polystyrene foam to avoid entangling of hooks and lines.

3.2.3. **Fish traps (Minnow traps/Fyke nets)** (Figs 1C, 2)

Operation

- Small portable wire or net baskets.
- Traps with wings designed to guide especially small demersal and/or benthic species into the trap.
- Fyke nets are large hoop nets that act as funnels to trap swimming fish.

Specifications

- Either small foldable net or wire traps with internal funnels leading to a collection chamber.
- For small species, up to 60 cm length and 30 cm opening diameter with small mesh size.
- For large (demersal/nocturnal predatory) species, larger traps with 5-20 mm mesh size, 2 m or longer with opening diameters up to 75 cm.
- Larger traps are available commercially or from local fishermen but more difficult to handle and normally inappropriate for scientific sampling (these can be set by the collector or can be purchased from fishermen with existing nets – the benefit here being talking advantage of local knowledge and skill).
- Either with two wings (up to 3 m length) which are attached to one trap, or two traps which are attached to one wing.

Application

- Usually placed with or without bait in the shallow, calm or low current water near the shoreline.
- Assembled and placed before dusk and removed in the early morning.
- Traps and attached wings can be placed under overhanging vegetation, littoral zones and in front of weeds.
- Fyke nets may set at the intersection of smaller creeks to block the free passage.
- Traps should be monitored regularly for (nocturnal) predators.

Fig. 2. Wagenia fishing with large bamboo fish traps in the Congo rapids at Kisangani (Democratic Republic of Congo). (Photo by U. Schliewen).

3.2.4. Fishing using ichthyocide (Rotenone)

Operation

- Traditional fishing method employed from indigenous tribes *e.g.* in Africa and South America.
- Utilising the extract from roots of certain plant species (especially those belonging to the genus *Lonchocarpus* and *Derris* or *Tephrosia*, the latter used locally in many villages in the Democratic Republic of Congo).
- Pulverised roots are placed in the water to release the active ingredient.
- Depending on the affected area, needs 5-10 persons with dip nets to collect the fish.
- Rotenone fishing can be a useful alternative in inaccessible habitats, SCUBA collections in deeper water and for the collection of cryptic and hole dwelling, otherwise inaccessible species.

Specification

- Commercially used broad-spectrum insecticide, pesticide and piscicide.
- Available either as fine brown powder or emulsified liquid.

- Classified as IATA air dangerous good (toxic, Class 6.1).

Application

- Powdered Rotenone must be solved in water using an emulsifying agent (usually detergent).
- Liquid Rotenone can be applied directly but should be diluted 1:10 first.
- After mixing, Rotenone is easily spread into the environment using smaller containers like plastic bags, bottles or buckets.
- Affected fishes show suffocating symptoms and either turn gaspingly to the water surface or fall to the bottom.
- Fishes should be euthanased in an anaesthetic immediately after capture (prior to death) to avoid further pain and irreversible abduction of the lower jaws and opercles (results in poor quality of preserved specimens).

Toxicity

- Extremely toxic to insects and aquatic life including fish – easily absorbed through the gills or trachea.
- Interrupts the electron transport chain in the NADH complex in mitochondria of aquatic animals and insects.
- Has only minor and transient environmental side-effects.
- Low toxicity to humans or higher vertebrates.
- Poorly absorbed by the skin and gastrointestinal tract of mammals (see Appendix for further information and follow link for MSDS provided in section 7, internet based information).

Remarks

- Rotenone is the most effective tool available because only small quantities are necessary and well suited for small scale sampling of cryptic, hidden fishes or shoreline fish communities.
- Application area should be free of thicker mud deposits with sufficient water visibility to allow easy detection and effective collection of those specimens falling to the bottom (specimens are easily covered by disturbed mud and become soon invisible for collecting).
- Sampling locations with strong currents should be avoided or the habitat should have some mechanism of containing the spread of the rotenone.
- Spread of rotenone to adjacent areas should be prevented (areas of current flow affecting easily larger areas than required).
- In small streams or creeks, block affected area up- and downstream with gill nets prior to rotenone application to catch those specimens which flee from the rotenone or affected specimens that drift with the current.
- Great care should be taken in frequented areas of nearby villages.

- Rotenoning from shore, boat or SCUBA should be evaluated regarding efficient collecting of affected fishes from the environment.
- The environment should be evaluated with this in mind together with an estimation of the number of specimens that will be affected in order to minimize large scale effects on populations and the environment.

4. Sampling and fixation

This section covers only the collection and sampling techniques for freshwater fishes for scientific (zoological) purpose, *i.e.* tissue samples and fixing voucher specimens in the field for final deposition in Natural History Collections.

The handling time needed to set and retrieve the fishing gear, and subsequent time to sort, tissue sample and fix the specimens, is often underestimated. As a general rule, 1 to 2 minutes per specimen should be calculated for retrieval, tissue sampling and fixation. This adds up to about 6.5 hours for 200 samples processed. Experienced crews with highly efficient sampling workflows will need less time, untrained ones may need even more. Also, this time frame can be greatly increased by any ancillary sampling requirements such as measurement and photographing. Tissue viability and natural coloration will quickly fade. Both can be extended by keeping the fishes alive as long as possible or through cooling the freshly dead specimens with ice.

All of these factors should be taken into consideration when planning a sampling event as these may determine: a) the number of specimens that can realistically be handled (how many of them can be photographed and/or individualised and tissue sampled); b) the number of sites that can be sampled in a day. This is especially critical when collecting previously unexplored regions and faunas to ensure well-preserved, well-documented, straight specimens and individualised tissue samples. Poorly documented and/or preserved specimens resulting from overambitious sampling events are not only of limited scientific value but are a waste of time and money (Figs 4E,G and 5F).

4.1. Necessary equipment and chemicals

Table 1 gives a list of minimally required field equipment for the adequate sampling and fixation of fishes in the field. This list omits any recommendations for personal equipment (such as multi-tools, headlamps for night-fishing, tripod chairs, rain covers, etc.). Appropriate footgear for fishing is discussed above. This suggested list may need to be adapted relative to the planned sampling and depends on the duration of the trip, destination and mode of field transportation which may allow only a minimal subset of this gear.

As a general rule, all preservation and fixation chemicals (together with any other hazardous substances like Rotenone) should be kept strictly separated from each other as well as from other field gear (especially personal items) to prevent any risk of personal injury and also contamination of tissues samples by residual formalin or formalin vapours. Under humid climates, formalin vapours may condense during cooler nights inside the box containing the sampling gear and may adversely affect other gear and degrade tissue sample quality.

Dissection tools, documentation materials, DNA-vials, towels and additional plastic ware for DNA-vouchers should be packed in a durable (aluminium) container that will withstand the rigours of rough field conditions.

Additional gear may be necessary for specialised sampling routines such as electrofishing (protective boots and gloves) or for live fish maintenance during collection (tubs, oxygen supply) etc.

	Items/Gear type	**Specifications**	**Remarks**
Documentation	pre-numbered field lists or field book	water resistant laser or ink jet print	numbering coherent with fish-ID tags and vial numbers
	pre-numbered field tags	water and formalin-resistant	*e.g.* paper printed or numbered plastic tags
	paper or transparent paper (2-3 sheets)	should be acid free	for additional location data to be included to the preserved specimens
	2 graphite lead pencil or graphite monolithe pencil	with graphite lead	industrial polymere lead pencils are made of coloured polymers not waterproof and rub off
	2 pigment ink pen	water & alcohol resistant pen	*e.g.* EDDING 1880 profipen, Securline MarkerII
	1 GPS device	positioning	should allow positioning to nearest 5 m and should receive satellite signals in forested areas
	1 camera	analog or digital	documentation of life colouration
	photo cuvette/photo aquarium	30 x 5 x 10 cm	documentation of life colouration
	(negative/slide) films or memory cards	storage medium	sufficient amount
Fishing gear (minimum) – non electrofishing	1 pole/beach seine	mesh size max. 10 mm	
	2 gill nets	monofilament nylon net, mesh size 10 mm	for small species
	2 gill/enmeshing nets	monofilament nylon net, mesh size 20-60 mm	for large species, mesh size depending on target species

	2 aquarium nets	20 x 10 cm	for juveniles and to sort the catch; 2 nets per person for snorkeling
	1-2 frame nets	60 x 40 cm with a 30-40 cm deep bunt	preferably collapsible
Fishing gear (optional) – non electrofishing	1 cast net	mesh size 6-8 mm, with bottom pockets, diameter 4-8 m	net diameter depending on the cast netting skills
	2-3 minnow traps or fyke nets	fine mesh, with funnels and collection chamber	trap should not exceed 30 cm diameter, wing not 3 m
	1-2 longlines	20 hooks/20 m line with small barbed hooks and strong hooklings	application either from shore or boat (requires additional anchors & buoys)
	angling/hook & line	1 spool and several small barbed hooks	especially in third world countries for fishing kids
Electrofishing gear	electrofishing device	see 3.1	suited model size and generated power dependent on habitat conditions and water conductivity
	anode rod with electro-shock dip net	anode ring with dip net (mesh size 6-8 mm)	electric field depending on the ring size; large ring (30- 40 cm) = larger but more diffuse field; small ring (20 cm and less) = more concise field
	cathode cable	braided copper cable	standard length 2-3 m; length might be adjusted (see under 3.1)
	1-2 dip nets	firm net ring, diameter ca. 30 cm	1 dip net per person (except for the electrofisher and operator of the device)
	2-4 buckets	10-20 l	for collecting the catch during electrofishing
	and/or 1-2 tubs	80-90 l	for collecting and holding the catch during electrofishing

	Electro-shock low voltage gloves	size depending on the persons	natural rubber gloves for use on circuits of up to 1000 volts
	Wellington boots/hip waders	size depending on the persons	
Preservation gear	1 set of dissecting tools	1 fine scissors 1 re-usable scalpel 2-3 sharp blades 1 medium forceps (serrated tips, manipulation of formalin vouchers) 1 probe 1 box of needles	minimum equipment list; two separate sets of dissecting tools for manipulation of tissue and formalin samples are mandatory to prevent formalin contaminations of DNA tissues; larger fishing crews should operate with at least two dissecting kits
	4% aqueous buffered formalin solution	10-20 l; ca. 1:9 dilution of concentrated formalin (37%)	be sure the formalin is buffered, or add additional buffer to keep the pH stable (especially in hot climates); 1 l 37% formaldehyde in solution as back-up and for injection of large specimens
	1 syringe (with strong needles and spare needles)	50 ml	for formalin injection of large specimens
	anaesthetic	minimum quantity: 2 l in solution	carry sufficient additional crystalline anaesthetic as back-up
	2-3 towels		to remove adhesive fish slime from the hands after sorting the catch (contamination risk) and to padding the photo aquarium for transportation
	multi fold sanitary paper towels (1-2 pack)	recycling quality, as supplied in towel dispensers	paper towels in recycling quality remain stable if soaked with fixative solution (compared to toilet paper or kitchen towels)

	2 square plastic food containers	30 x 20 cm (as large as available)	minimum number; should be as leak proof as possible (rely on quality products) to prevent formalin leakage
	1 aquarium net	20 x 10 cm	to dip the fish in the anaesthetic
	5-10 PE plastic bottles	1-2 l	for direct fixation without prior pre-fixation of small specimens
	1 measuring jug	500-1000 ml	to pour the fixative into the fixation containers
	2 plastic kegs (optional)	20 l	round plastic kegs with red screw-on lids with air and watertight O-Ring rubber gaskets, UN-X approved for storage and further fixation of pre-fixed specimens
	cheese cloth/cotton cloth (optional)	sufficient	for enwrapping preserved specimens for transportation
	2 plastic kegs	60 l	as specified above, one for formalin, one for anaesthetic storage,
	2 plastic buckets (optional)	10-20 l	for transportation/sorting of the catch
	2 large plastic tubs (optional)	ca. 100 l	for holding of captured fish; required for storage during electrofishing
Tissue sampling gear	separate set of dissecting tools	separate blades for re-usable scalpel 1 fine scissors 1 fine forceps (with smooth tips)	manipulation of DNA-tissues
	2.0 ml self-standing microtubes	200-400	pre-numbered, prefer renowned high-quality brands
	1-2 storage boxes	100-200 place boxes	large boxes are easier to handle compared to small 81 place boxes
	toilet paper	1 coil	required for cleaning of scissors/forceps after each sampling

	centrifuge tubes	50-100, self-standing form	for preservation of small fishes as DNA-voucher
	96% distilled ethanol	1 l	as back-up and to fill the centrifuge tubes

Table 1. Field and preservation equipment.

4.2. Documentation – general aspects

The precise, accurate, detailed documentation of all associated data is of vital importance for any biological sampling. The more information is collected on the geographic, taxonomic and habitat characteristics of specimens, the more valuable these specimens become to the scientific community. Ideally, exact location should be pinpointed with the aid of a GPS unit. This may entail single point data, start and end points of a transect or corners of a rectangular area. If a GPS unit is not available or practical, precise written, descriptive locality information becomes that much more important.

At all times it is crucial that all associated data (geographic, taxonomic and habitat) be stored together with the samples to ensure correct interpretation and matching of this data to specimens. All field notes, labels and ancillary documentation should be taken on weather proof, alcohol and formaldehyde resistant paper and with good quality ink or pencil (see Table 1). It is helpful to draw a picture of the sampling location highlighting features of interest (vegetation, current direction, physical features, etc.), habitat specifications and exact sampling location(s), gear used and species collected. As collecting gear or sampling site might be very specific for species or sizes, the precise documentation of the gear types used at the respective habitats adds valuable biological and ecological information to the collected specimens. This allows conclusions on daytime and habitat preferences of species or different live stages of the species. A comprehensive list (but not be limited to) of information to be collected is included in the Appendix (Documentation of Collecting Event). Any semi-accurate information must be omitted, *e.g.* habitat or locality information for specimens purchased from the fish market (see comments in section 3.1, fish market), unless the exact location is known.

4.3. Landing, sorting and euthanizing the catch

Any catch must be landed carefully. This applies especially to landing net collected species where entangling may cause damage to the specimens, *e.g.* curling beach seines with the catch and debris and stones up the beach. Instead leave the net gear (and the fishes) in the shallow water (being sure to elevate the edges of the net to prevent escape) and retrieve the specimens as quick as possible. This may be unavoidable in the case of gill nets that are left in the water for extended periods. Fishes should be removed from entanglements as carefully as possible to ensure no further damage is done to the specimen. Specimens should always be removed head first to avoid fin damage or de-scaling. Care should be taken with spines as they can damage the net, the fish and the collector. Bony spines (and their serration) are valuable

diagnostic characters for many species. Be aware that some species have poison glands associated with bony spines which can cause painful injuries or numbing and the affected area may cause dizziness or even severe allergic shock.

Living specimens recovered from electrofishing or retrieved alive from fish traps or hooks might require temporary holding in large buckets or tubs for later euthanasing or photo documentation of live colouration. The water temperature should be ambient and the containers placed in the shade protected from direct sun. Covering the container reduces stress and prevents escaping. Overcrowding should be prevented and the water should be exchanged at regular intervals to reduce physiological stress. Additional (pure) oxygen-supply from oxygen-bottles may be mandatory (*e.g.* for holding during electrofishing in several European Countries).

Rapid sorting of the catch is crucial:

- to obtain an overview of the number of included species;
- to separate those specimens required for DNA sampling;
- to release or fix the remaining specimens.

Especially in hot climates, specimens die rapidly and this immediately starts autolysis of the guts which may compromise tissue removal, particularly in predatory and herbivorous species. Sort the catch according to species or genera required for sampling. Dead specimens are either set aside for later tissue-sampling or immediately fixed in formalin. Specimens should be placed in appropriately sized containers where the total ratio of animal tissue to fixative does not exceed 1:3, enabling the specimens inside to float. Small dead specimens (up to 10 cm total length) for formalin fixation are immediately collected head first either into 50 ml centrifuge tubes (Figs 3D & 4H) or PE-plastic bottles (1-2 l). Plastic containers (buckets, bottles, tubes, jars, etc.) should be constructed of UV resistant material to ensure they maintain their integrity. Insert a folded (to prevent rubbing and removal of lettering), water resistant label with sampling data written either with pigmented ink or pencil and fill the bottle to the top with the fixative solution (to prevent desiccation of specimens extremities). Leave the container horizontal for at least 1 hour to ensure that the specimens remain straight and do not bend during pre-fixation. After this pre-fixation, the container can be turned in upright position for thorough fixation of specimens (1-3 days, depending on specimen size, Figs 5D & 5F). Remaining specimens should be kept alive and fresh as long as possible to keep (and document) their living colouration. Dead specimens can also be placed on ice to maintain tissue integrity and colouration, especially if photographing or additional treatments are needed. Specimens may be euthanized immediately before tissue sampling and fixation by (repeated – if necessary) exposure to a suitable anaesthetic for few (5-10) seconds (see 2.4 for anaesthetics and doses). Sort the fish to be tissue sampled according to species as best as possible, since carrying forward the same species information into the tissue sampling lists is much more convenient and less time consuming. Euthanize only enough specimens as can be processed by the sampling crew in a single session. Depending on the species and fish size, if

opercular movements have ceased for ca. 5 minutes, the fish can be considered to be dead. Be aware that air breathing species, such as some Cyprinids, many Silurids, Gouramis and Lungfishes, or fishes with low respiration rates (*e.g.* during winter), are less perceptible for water soluble anaesthetics and may require repeated exposure to be euthanased. The live colouration should be documented (written description or photographed) prior to or shortly after euthanasing, but before fixation, since the pigment cells will relax and expand turning specimens dusky after treatment.

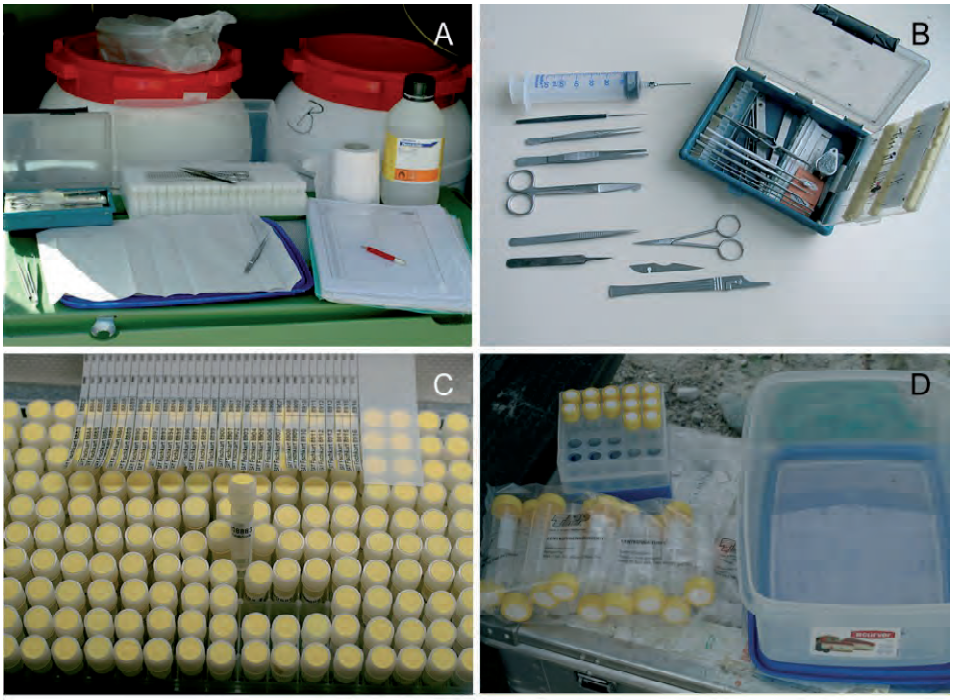


Fig. 3. Preservation and dissecting gear. A. Mobile tissue sampling station and documentation in a van: right, keg drumstores anaesthetic; left, 4% formaldehyde solution; euthanased specimens are sorted and tagged on fresh paper towels to minimise mucus contaminations, dissecting tools cleaned with absolute ethanol and toilet paper; B. Dissecting tools including two smooth forceps, scalpel blades and a small scissor for tissue sampling (bottom), different sized probes and needles for raising and fixing fins, larger serrated forceps for manipulating formalin specimens, large scissor and Luer Lock syringe for penetration of the abdominal cavity of larger specimens; C. Pre-numbered 2 ml storage vials (NUNC), 200 vials per rack, with corresponding gill tags (smooth tracing paper 110 g/m² printed with HP DeskJet 600 with original HP Cartridge no. 29); different sizes of the same numbers for tagging of smaller (first two numbers only) or larger (complete tag) specimens; D. Self-standing 15 ml and 50 ml centrifuge tubes (TPP) for fixation of small specimens and fixation container (commercially available plastic food container). (Photos A, B, D, F by S. Beyer, all other photos by D. Neumann).

4.4. Tagging of specimens and preservation of DNA-tissues

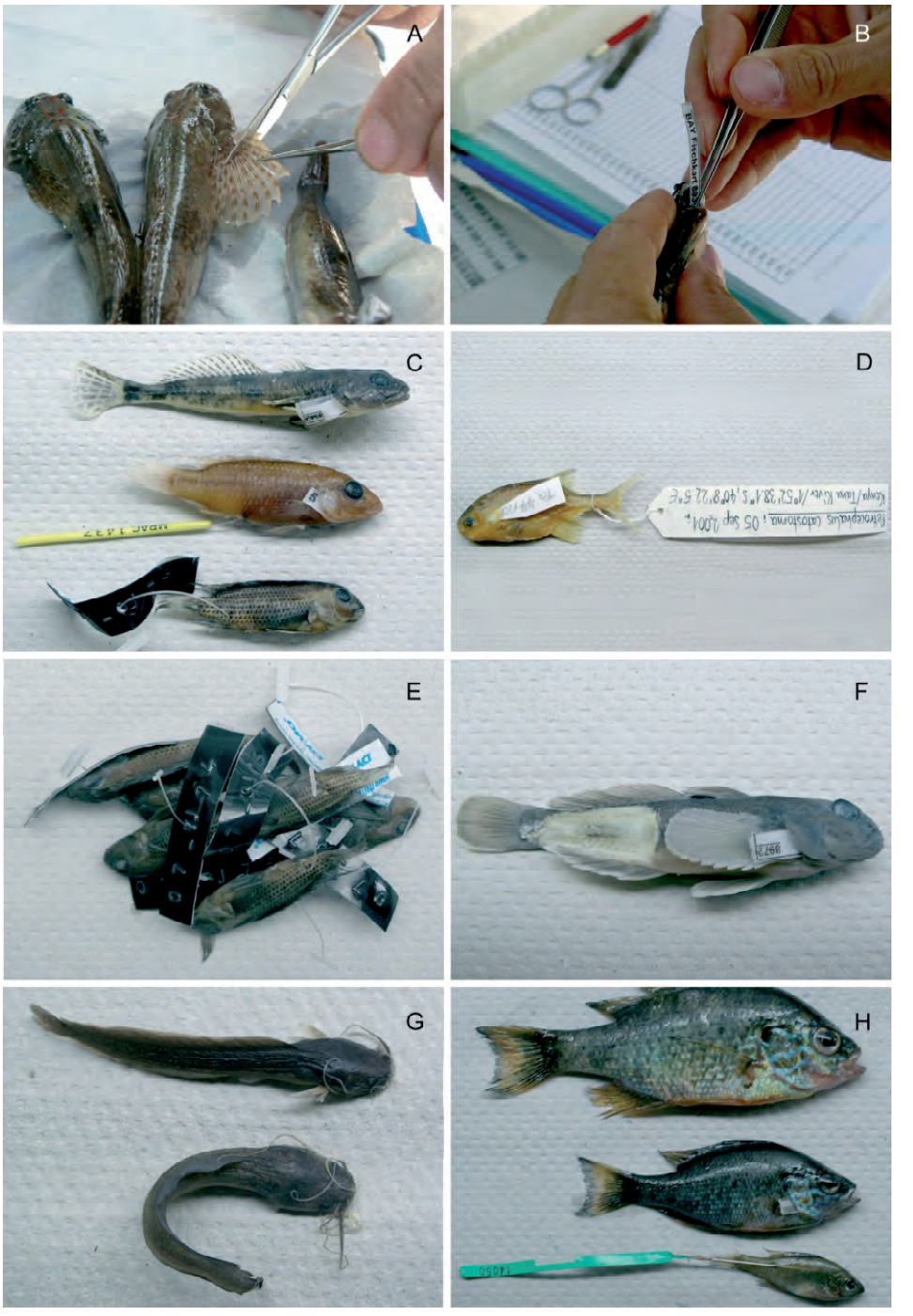
While formalin fixation varies only in relation to specimen size, tissue sampling necessitates exact and clean working to avoid cross-contamination of tissue samples, and should be done by a well-trained crew to speed up tissue sampling. Efficiently tagging and sampling of specimens requires two people, one for preparing, sorting and tagging, and the other for tissue sampling. At all times the link between specimen and tissue should be maintained both in field notes and through tagging of specimen and labelling of tissue tube. Tissue sampling requires a separate set of dissecting tools to avoid formalin contamination of tissues (for basic tissue sampling set up and requirements see Figs 3A-D). A set of freshly dead specimens (4-6, depending on size) are placed (sorted to species) with the head to the right on multi-fold sanitary paper towels to absorb residual mucus (Fig. 4A). Tissue tags can either be applied directly into the muscular tissue (Fig. 4C bottom, 4H bottom) or into the anus (Fig. 4C middle) using commercially available ribbon tags, t-end pins or similar (Fig. 4C), poly streamer tags (Fig. 4H, bottom) or tied through the gills (Figs 3C, 4B). Specimens are always tagged on the right side of the body, regardless which tag type is applied. In some species they may also be tied around the tail (Fig. 4D) ensuring that the tag will not work itself loose or become disassociated from the specimen. Tubes should be labelled with pencil or indelible ink, or both with information linking the specimen and tissue (Fig. 3C). Insertion of labels into tubes should be avoided to prevent contamination of tissue. The sampling crew should verify species and tissue tag numbers and tissue vial numbers during sampling to avoid mistakes during handling.

All tissues are sampled always from the right hand side of the specimen as the left hand side is traditionally used for measuring and photography. Muscle tissue is preferred to prevent having to gut the specimen for heart or liver tissue. Muscle tissue is usually removed from above the pectoral fin or on the caudal peduncle and should not alter the contour of the specimen (Fig. 4F). Muscular samples from the right abdominal region (behind the anus) are preferred if specimens have been dead for any length of time or if they have started decaying.

Fin clips are commonly sampled but may yield less DNA (quality and quantity). Usually only the lower portion of the pectoral fin is sampled, so that the total fin length remains unchanged and fin rays are still countable (both are diagnostic characters) (Fig. 4A, 4H top specimen). Depending on the specimens size, it may be necessary to cut the pectoral and pelvic fin, including the muscular fin bases (Fig. 4H, specimen in the middle), or to abduct the complete caudal peduncle (if more than one specimen is available) to receive enough tissue. The latter method should not be the first choice, because identification especially of small species under field conditions is extremely difficult, and recognising that the only minute specimen of a potentially new species lost its diagnostic character together with the caudal peduncle thereafter will neither improve the specimen, nor its condition. Cutting off bony spines has to be avoided for the same reasons, since most spines bear diagnostic characters and are essential for species identification as the left spine might be broken or missing (Fig. 4G).

It is best to use a new disposable scalpel blade for each tissue extraction, or to clean the scalpel blades or scissors after abducting the fins to prevent cross-contamination from one specimen to another. For this purpose, wipe and clean all tools (scissors, forceps, and scalpel) after each processed specimen best with 96% ethanol. If 96% ethanol is not easily accessible in the field, clean thoroughly with dry towel or toilet paper. While contamination between of allopatric species separated for a long period on a geologic time scale might be detected, it is impossible to detect cross-contaminations of just recently split or hybridising species. Same applies for any population genetics. The forceps for tissue manipulation should have smooth tips rather then serrated ones, to allow better cleaning of the tips and to avoid contaminations from residual mucus adhering to the serration. Excess mucus or debris should be removed from the tissue extraction site using paper towels or similar after which scales should be removed and the area cleansed (using 96% ethanol).

Fig. 4 (next page). Tissue sampling and tagging. A. Fin sampling of the lower portion of the right pectoral fin of euthanased sculpins; paper towels remove residual mucus; B. Gill tags should be folded with a small hook at the proximal end of the tag which is applied between 1st and 2nd gill arch (at least 2/3 of a gill tag should be covered – and secured – from the opercle); C. Application of different tags (from above): gill tag printed on smooth tracing paper with HP 600 DeskJet printer, T-anchor tags (Hall Print) applied into anus are firmly attached by piercing the gut canal (mind not to damage genital papillae during tagging to allow sexing of specimens thereafter), DYMO-tags applied with t-end pins and commercial tag guns (badly damaging especially small specimens); D. Small Mormyrid with handwritten tag (pigmented ink on durable paper) tied to the tail (museum gill tag easily lost and inappropriate for field conditions); E. Coiled up specimens removed from a museum jar: large t-end pins and even larger rigid plastic tags fixed to small specimens impede straight fixation and further damage them during museum storage; note that the imprint of DYMO-tags gets illegible in alcohol after few years; F. Muscular tissue samples should be removed from the tail (behind the anus) without altering the contour of the specimen. G. Diagnostic pectoral spine removed in the lower specimen for tissue sampling. H. Abduction of the fin base in smaller specimens might raise the DNA-content of the tissue sample; alternative PE-plastic streamer tag attached to a needle, applied in rostral direction into the muscular tissue causing minimal damage to the specimen (Caution: for museum storage plastic tags should be replaced because of potential corrosion from denaturing agents and/or high alcohol concentrations). (All photos by D. Neumann).


A
B
C
D
E
F
G
H

Tissues should be placed immediately into labelled tubes (preferably good quality, self standing, gasketed tubes), filled with 96% ethanol and tightly sealed (Fig. 3C). Cryo-tubes, which are especially designed for cryo-storage at deep temperatures, might cause problems under hot climates because of high evaporation losses. Repeated cases of evaporation losses of 50% or more have been reported. Be aware that in such cases the concentration of the residual ethanol may be far below 96%, since ethanol below 80% evaporates as pure ethanol. Tubes with 50% evaporation losses are inappropriate for tissue preservation and should be discarded since the ethanol concentration might have dropped below 40%. Prefer 96% distilled instead of chemically dried ethanol (concentrations of 99% or higher) as residual low boiling benzines used for drying the ethanol might degrade the DNA. The amount of tissue in any tube should not exceed one third of the total volume to allow for efficient and rapid preservation of tissues. If not avoidable, replenish with new ethanol after 2 hours. Tissue may also be cut into smaller pieces or macerated to facilitate this process.

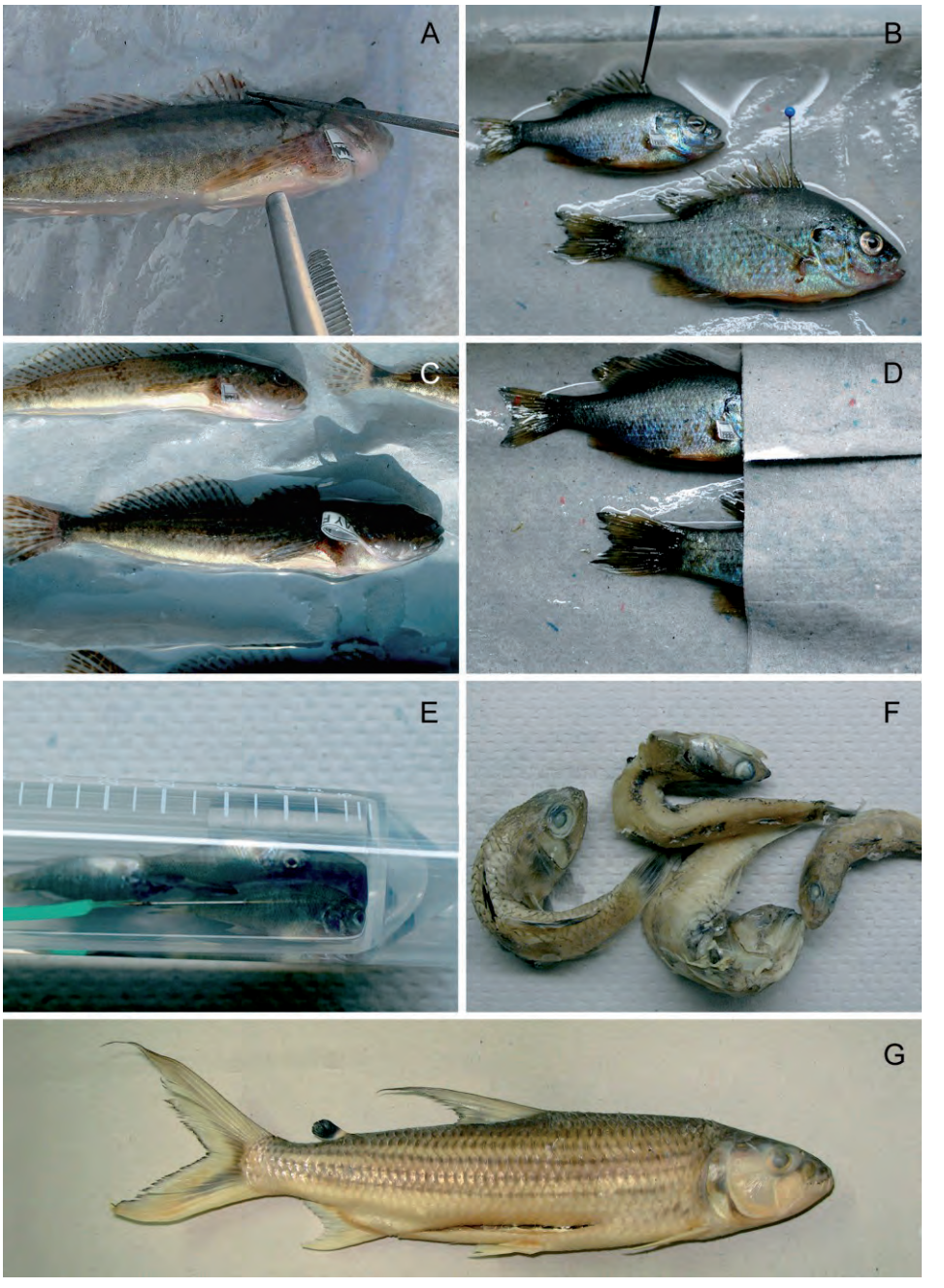
If no field tags are available for some reason, species should be sampled in order and by size to provide a mechanism of verification. For smaller specimens, whole specimens may be placed in tubes as tissue voucher but either photographs should be taken as vouchers or other specimens undoubtedly of the same species can serve as surrogate vouchers (not preferable).

4.5. Formalin fixation of specimens

Fixation and preservation is not the same. Preservation should only follow adequate fixation. Fixation stops autolysis by cross-linking and degrading proteins into amino acids by the formation of covalent bonds and coagulates cell contents to insoluble substances, whereas preservation alters the hydrogen bonding pattern and preserves the specimen by de-hydrating it (Simmons, 2002). Therefore, DNA-extraction of ethanol preserved tissues is possible (with DNA quality strongly depending on quick and efficient tissue dehydration), while extraction from formalin fixed tissues yields only short degraded gene fragments, depending on the number of cross-links and bonds which prevent the uncoiling of the DNA-Helix and thereby disabling the bonding of DNA polymerase.

The fixative should penetrate the specimens as rapidly as possible to prevent decomposition, especially of the guts and their contents. This initial or pre-fixation should be done within 10 minutes of the specimen dying.

Fig. 5 (next page). Fixation of fish. A. Prevent fusiform specimens from turning during prefixation; B. Prefixation of unpaired fins requires roughly 5 minutes; C. Orientate specimens in the one direction with sufficient spacing; D. Specimens stacked in different layers head on tails separated with formalin soaked paper towels; E. Small specimens (tissue sampled or ethanol vouchers) fixed head first horizontally in 50 ml centrifuge tubes; F. Nearly impossible to measure these bent specimens and useless for other morphological approaches; G. Opening abdominal cavity of large specimens allows quick fixation of the guts. (All photos by D. Neumann).


A
B
C
D
E
F
G

This 10-min time frame is crucial and requires a disciplined and experienced DNA-sampling crew and fixation routine.

The most common fixative is a 4% (or 3.7%) aqueous formaldehyde solution; in the tropics and for larger specimens, a higher concentration of 10% should be considered for quick pre-fixation (= 1:4 dilution of 37%, concentrated formaldehyde solution with tap or river water). Use only buffered, methanol stabilized formaldehyde solution (Simmons, 2002) for fixation to maintain a stable pH range as formaldehyde is unstable and oxidises in water into formic acid and to prevent decalcification especially of the often minute and fragile bones of fish skulls. While decalcification begins at a pH of 6.4 and below, clearing of soft tissues may already start at a pH of 7.0! Additionally, unbuffered formalin causes subsequent problems during later museum storage. Omitting the buffer from the formalin will shift the pH inside collection jars to acidic ranges. If you are dependent on locally available, unbuffered formalin because of transportation limitation (*e.g.* IATA aviation restrictions), this can be buffered through the addition of Sodium Phosphate Dibasic Anhydrous (CAS 7558-79-4) and Sodium Phosphate Monobasic Monohydrate (CAS 10049-21-5) in a ratio of 6 grams and 4 grams per litre respectively, or by adding a carbonate buffer; even chips of marble or limestone will help. Be aware that it might be necessary to add more then 6 (4) grams per litre of buffer, if you are depending on river water with low (acidic) natural pH values (*e.g.* streams draining rainforest or granite soils) for dilution of higher concentrated formalin to receive a 4% aqueous formaldehyde solution.

The diffusion rate of formaldehyde through the animal tissue is crucial and this may be slowed by thick or swollen mucus layers (*e.g.* in eels or sculpins) or size of the specimens. Simple formalin permeation (placing specimens directly into the fixative) is sufficient for fixation of small specimens up to 10 cm. Cover the bottom of the fixation container with 1-2 paper towels and a small amount of formalin, preventing the specimens from floating. This should allow the specimens to be fixed in a natural position. Better quality specimens may be obtained by raising the dorsal and pectoral fin with a probe thereby fixing the spines against the formalin soaked paper towel together with spreading the caudal fin. This improves counting of spines and soft rays of preserved specimens. If necessary, hold the raised spines in place with a probe or fix one of the first spines with a small needle. The minute muscles responsible for the fin movements are fixed within 5-10 minutes (Figs 5A-B). This may not be possible for all specimens due to time constraints.

Gill covers and branchiostegal apparatus should be in a normal position, the mouth should be slightly open, and the jaws should not be abducted. If a specimen has suffocated prior to relaxation in the anaesthetic, hold the fish and simultaneously keep the gill covers closed with one hand, while carefully closing the mouth and setting the branchiostegal apparatus back into a normal position with the other. Cut a finger off a disposable glove, push the specimen into the closed end and place it carefully into the fixative. Locked pectoral spines of catfishes should be released by pulling the spine carefully backwards (caudal direction) and turning it (beware of serrated or poisonous spines). All specimens which are placed in one layer into the fixative should be orientated in the same

direction. Take care that the new layer is sufficiently moist with formalin and place the next batch of specimens in the opposite direction (heads to tails) and raise the new fins by placing them on the bodies of the previous layer. The vertical distance between single specimens should be wide enough to unfold the caudal fins (Figs 5C-D).

This method of stacking the specimens in different layers of paper towels has several advantages:

- Pre-fixation and fixation can be done in the same container while limiting the necessary amount of formalin and minimizing the amount of formalin vapour.
- Specimens inside the layers will support each other resulting in straighter preserved specimens for natural history collections (compare Fig. 5F).
- Different sampling locations can be separated inside one fixation box (in this case pay attention to fold the outer ends of the towels upward to prevent that single specimens slip from their layer and mix with other locations).
- During transportation the arrangement in layers stabilises the specimens during fixation.
- The layers will also reduce movement of free formalin solution inside the box which stabilises the box during transportation and lowers the risk of potential spillage.

For specimens ranging from 10-20 cm, the permeation method is sufficient for pre-fixation only (10-20 minutes). After this time, the specimens need to be immersed in 4% formaldehyde solution to ensure high penetration rates (*e.g.* by stepwise filling the fixation box to the lowest layer with formalin).

For good fixation results and efficient penetration of specimens larger then 20 cm, the fixative may need to be injected into the body cavity. Assure that formalin/fixative is injected only into the cavity and not into the muscular tissue, which leads to intramuscular tissue rupture and formalin swelling of the tissues. Penetrate the abdomen laterally through of the anus or the belly near the (scaleless) base of the pelvic or pectoral fins with a syringe and needle (preferably a Luer Lock syringe and needle to prevent the needle from being propelled off the end of the syringe due to pressure build up inside the specimen's belly). Caution: To prevent eye damage through exposure to formaldehyde always wear protective eyewear (safety glasses or goggles) and turn the specimen away from your face when injecting and removing the needle.

Specimens larger than 30 cm should either be injected with 10-37% formaldehyde solution (37% for specimens larger than 50 cm) or the abdominal cavity should be opened with a sharp scalpel. Insert the scalpel into the anus (blade in parallel direction with the inner body cavity), cut 2-3 scale rows upwards in a dorsal direction and then turn in a rostral direction and parallel to the ventral border of the belly so that the natural contour of the specimen remains unchanged (Fig. 5G). Care should be taken to avoid damages of the genital papilla to ensure sexing of the specimen and to cut only the lateral right side of the body to keep the pre-anal measurements of this specimen.

Large specimens need to be placed in larger UN-approved, tight closing plastic kegs. For fixation it is important to leave the drums in a horizontal position for at least a day for good fixation results and to avoid deformation of specimens. For transportation, place the keg horizontally in a plastic box to collect any potential leakage and secure against rolling.

4.6. From fixation to preservation

Small to medium sized specimens should be left in sufficient formaldehyde solution in the fixation box for at least one week. Larger specimens should be checked periodically to ensure adequate fixation of the entire specimen – the belly should be firm, muscular tissue should be moderately hard, leaving no thumb imprint behind after manual inspection. If the gut contents of specimens (mainly herbivorous) start to decay, fermentation may cause gas to build in the gut cavity distending the specimen. In this case, the abdominal cavity needs to be opened and washed out thoroughly. Care should be taken when opening the abdominal cavity of such a specimen to prevent chemical eye burns and this can be done underwater to alleviate any pungent smell emanating from the specimen.

After fixation of specimens, the transfer to the preservation fluid has to be done in several steps to remove residual formalin from the specimens, and to avoid dehydration or cell rupture. First, wash the specimens by rinsing in water or by immersing in water and exchanging the water several times on a daily basis. This procedure should be repeated, until no or only a moderate formalin smell is perceptible. Then transfer the specimens into a 20% ethanol solution for 1-2 days (for specimens up to 5 cm), one week (specimens up to 20 cm) or 1-2 weeks (larger specimens), and repeat this procedure with 40% and 60% solutions before finally transferring the specimens into 70% ethanol. From personal observations, specimens originating from high water conductivity environments (especially European Cyprinids) seem to be highly susceptible to ethanol dehydration and thereby should not be transferred directly from formalin into 70% ethanol to avoid shrinkage.

5. Packing for transport

Returning samples from the field, including DNA-tissues as well as formalin preserved specimens might give rise to some unforeseen difficulties. Specimens should therefore always be packed in such a way that: a) they are not damaged during the transit; b) the specimen can be stored under stable conditions inside the containers for several weeks. This might be necessary if an airline refuses to carry formalin samples and you are forced to return the samples by surface mail from third world countries.

5.1. Transport on the road from the field site

Packing requirements of the specimens strongly depends on the road conditions.

5.1.1. On paved roads

Preservation and fishing gear has to be packed in such a way to prevent leakage from formalin containers and personal injury in case of emergency braking. All field gear should be secured in the trunk with straps, as for oxygen bottles and especially larger drums containing formalin or anaesthetics. Formalin containers should be placed in larger plastic or aluminium boxes to avoid any formalin leakage into the trunk particularly for bendy roads. Formalin and ethanol fixation gear always has to be stored in separate boxes to avoid any formalin contamination of the ethanol gear. Square plastic food containers are never 100% leak-proof and should be filled only to 5 cm below the top to minimise potential leakage. Formalin vapours emanating from the trunk into the cabin might cause dizziness or sickness to the driver. Plastic keg drums with formalin specimens should be carried horizontally or nearly horizontally (*e.g.* if a drum is placed and strapped into a tub) to avoid bending of only weakly fixed (larger) specimens inside the drum.

5.1.2. On gravel roads or rough tracks

For the transport of specimens for long distances and days over rough roads or dirt tracks, the packing of specimens requires additional precaution to avoid physical damage to them. Smaller PE-plastic bottles and kegs should be filled to the top with fixative leaving no air inside, to keep specimens in stable condition inside. Any free air space inside will shake both fluid and specimens vigorously after every road bump likely damaging the fins and abrading specimen-ID labels or DNA-tags. To protect fixed specimens in a keg or drum, wrap medium to large specimens in formalin soaked cloth. Each wrap should include only (consecutive series of DNA sampled) specimens from the same location to allow identification of specimens in case single field tags become illegible. Small fixed specimens can be easily separated and secured in disposable tea bags and several of those bags should be wrapped in cloth. For packing and returning material from foreign countries, decant the formalin and separate and pack the specimens in the same way. Additionally, single wraps inside the kegs may be packed inside aquarium bags and sealed tightly with rubber bands to minimise the risk of formalin leakage from the drums during transport. This method is not suited for pre-fixed or only weakly fixed specimens because the weaving pattern of the cloth (or specimens itself!) will imprint into the epidermis of the specimens!

5.2. Transport as carry-on luggage on board on aircraft

Carrying pure alcohol in hand or checked luggage onboard an aircraft is strictly prohibited (IATA, 2009). This applies for all DNA-samples *e.g*. placed in 2.0 ml tubes. Salt-based DNA-buffers (*e.g*. DMSO) or buffers with a volume less then 24% ethanol are not regulated. Alcohol based buffers should be referred to as “DNA-buffer” only when entering or leaving Islamic countries to avoid problems at customs. Aqueous formaldehyde solutions with less than 25% formaldehyde are not regulated under dangerous goods whereas solutions with more than 10% are classified as aviation regulated liquid (UN 3334). Concentrations less

than 10% are not regulated at all. To comply with the current IATA regulations, specimens should be wrapped in formalin (4%) soaked moist cheese-cloth, sealed in PE-plastic bags inside the drum as described above, and must be packed leak-proof in and best in UN-approved plastic kegs or drums. Note: There will be new regulations and amendments for shipping and transport of natural history specimens on board of aircraft in the 52st Ed. of the IATA Dangerous Goods Regulations affective on 1 January 2011 and perhaps in future; please keep yourself updated to comply with the regulations!

6. Acknowledgements

Most of the techniques outlined in this manual have been tested and adapted during the course of the project “Erfassung der Fischartenvielfalt Bayerns” (Species inventory of Bavarian freshwater fishes) 2003-2008 and various field work projects mainly in Africa conducted from ZSM staff (permanent and PhD students), sampling more then 10,000 individualised tissues and much more formalin specimens. The manuscript benefited from various experiences acquired during field sampling projects and/or input regarding field techniques from the following persons (alphabetical order): J. Freyhof (IGB, Berlin), M. Geiger (ZSM), T. Moritz, N. Pöllath, J. Schwarzer (ZSM), U. Schliewen (ZSM), E. Vreven (MRAC) and from various students deployed during the Bavarian species inventory. The manuscript gained from comments of two anonymous reviewers and especially from valuable contributions and critical comments from Andy Bentley (Ichthyology Collection Manager, University of Kansas) and Simon Moore (Senior Conservator of Natural Sciences, Hampshire County Council, Winchester). Without their help it would have been difficult to finish this manuscript under the given time constraints. Additional thanks for picture contributions to U. Schliewen (Fig. 2), M. Geiger (pictures in Fig. 1D) and Sebastian Beyer (Fig. 3A-B, D, F). All other photographs by D. Neumann.

7. References

IATA, 2009. Dangerous Goods Regulations, 50th Edition. International Air Transport Association, Montreal - Genf: 880 PP.

SIMMONS, J.E. 2002. Herpetological Collecting and Collections Management. *Society for the study of amphibians and reptiles, Herpetological Circular* 31: 1-153.

8. Further reading

For further information especially on net gear, electrofishing and fishing methods in general, the “Fish Collection Methods and Standards (Version 4.0)” is very valuable. This free Guideline issued by the Government of British Columbia (http://ilmbwww.gov.bc.ca/risc/pubs/aquatic/fishcol/assets/fishml04.pdf) can also be viewed (http://ilmbwww.gov.bc.ca/risc/pubs/aquatic/fishcol/index.htm) online.

9. Additional Internet based Information

CITES: http://www.cites.org

IUCN: http://www.iucnredlist.org

OIE: http://www.oie.int/eng/en_index.htm

How to throw a cast net: http://www.ausfish.com.au/castnet/

Aquatic Animal Health Code, Cpt. 5:
http://www.oie.int/eng/normes/fcode/en_sommaire.htm

Name	CAS-Number	Properties	Availability	Safety	IATA
Chlorobutanol	57-15-8	white, coarse crystalline powder	www.sciencelab.com VWR International	Irritant (eyes, skin) and extremely dangerous if ingested	not regulated
Ethanol (96%)	64-17-5	colourless liquid	www.sciencelab.com Sigma-Aldrich VWR International	Irritant (eyes, skin)	class 3
Eugenol	97-53-0	liquid oil		Irritant (skin), inhalation hazard; seek immediate medical attention in case of serious contact	may be regulated as UN 3334
Formaldehyde solution (10%)	5-00-0	colourless liquid	www.sciencelab.com Sigma-Aldrich VWR International	Irritant (skin), inhalation and ingestion hazard; carcinogenic, mutagenic and teratogenic effects (suspected or possible); classified developmental toxin to male/female reproductive system	concentrations of 10% and below may be regulated as UN 3334; concentrations of 10-24% regulated as UN 3334; less then 25% regulated as class 8 (UN 2209)
MS-222	886-86-2	white, fine crystalline powder	Sigma-Aldrich VWR International	Irritant (skin, eyes and respiratory system)	not regulated
Rotenone		brownish fine powder or dark	Sigma-Aldrich	Irritant (eyes and inhalation); after eye contact and ingestion seek	class 6.1

		brown fluid		immediate medical attention; Lethal oral dose (Human): 143 mg/kg	
Disodium Hydrogen Phosphate	7558-79-4	clear or white crystalline hygroscopic powder	www.sciencelab.com VWR International	Dust may cause irritation (eyes and respiratory system)	not regulated
Sodium Dihydrogen Phosphate	10049-21-5	clear or white crystalline hygroscopic powder	www.sciencelab.com VWR International	Dust may cause irritation (eyes and respiratory system)	not regulated

11. Appendix 2 - Material Safety Data Sheets (MSDS)

Chlorobutanol: http://www.sciencelab.com/xMSDS-Chlorobutanol-9923417

Disodium hydrogenorthophosphate:
http://www.chemicalbook.com/ProductMSDSDetailCB1242667_EN.htm

Ethanol: http://www.sciencelab.com/msds.php?msdsId=9923956

Eugenol: http://www.sciencelab.com/xMSDS-Eugenol-9924007

Formaldehyde solution (10%):
http://www.sciencelab.com/msds.php?msdsId=9924096

MS-222:
http://caligula.bcs.deakin.edu.au/bcs_admin/msds/msds_docs/Ethyl%203-aminobenzoate%20methanesulfonate%20salt%20(Sigma%20A5040).pdf

Rotenone:
http://caligula.bcs.deakin.edu.au/bcs_admin/msds/msds_docs/Rotenone.pdf

Sodium Dihydrogen Phosphate:
http://www.sciencelab.com/msds.php?msdsId=9925021

12. Documentation of Collecting Event

Collecting Event

- Field number (usually provided by the collector) *e.g.* XYZ-2009/01 (three-letter country code-year/sample number).
- Date of collection – start and end date if necessary.
- Gear used – type or combination of different gear types.
- Gear specifications – mesh size, depth, exposure time, voltage/current (as precise and detailed as possible).
- Habitat specifications referred to employed gear type – (gill net in front of weeds, kick-net sampling in riffles, etc.).
- Habitat conditions referred to collecting site (hard/soft/sand bottom).
- Full names of all collectors (first, middle and last names).

Locality

- Drainage – might be hard to discern *e.g.* for small streams or swamps.
- Geopolitical designation (country, state/province, county, etc.).
- Detailed locality string – usually in ‘named place’ and ‘location to named place’ format.
- Highlight any distinctive features like road and river crossings (indicate any distances by road or linear – *e.g.* 5 miles West of…).

- Any other geographic subdivision (State, Province, Department, Village or Township, County (?) etc.).
- Latitude and longitude.
- Geodetic datum.

Specimen

- Identification to family, genus or species level (if known, elsewise unspecific as *e.g.* as “Cyprinidae indet.”, “Barbus sp.” to allow re-identification of tissues in cases of erroneous sampling lists).
- Any distinctive characteristics – colouration, morphological characters as these may be lost during fixation and preservation.
- Size – in millimetres as standard or total length (SL or TL) or as disc width for rays etc. (can slow down specimen processing and can be done at a later time).
- Weight – in grams (often times not practical in the field and may be done later).

Habitat

- Habitat specifications – road side ditch, floodplain, swamp, forest stream, rapids, etc.
- Peculiar habitat conditions – leaf litter, deadwood, dense weed standings, boulders, etc.
- Climate – cloud cover, precipitation etc.
- Season – rainy/dry season, summer/winter.
- Stream type – white water, clear water, black water.
- Water and ambient temperature.
- Water clarity.
- Water pH.
- Water conductivity.
- Current strength and direction.
- Associated vegetation.
- Associated species not collected.

Acknowledgements

No less than 79 authors from 14 different countries have contributed to the two parts that constitute volume 8 of the series *Abc Taxa*. Their names, institutional affiliation and contact information are put under each chapter heading, while their short professional biography can be found at the back of the second part of this volume.

In addition, thanks go to Marta De Biaggi (Torino), Merijn M. Bos (Arnhem), Pierre Commenville (Nice), Vanessa Demanoff (Paris), Henrik Enghoff (Copenhagen), Olivier Gargominy (Paris), Fabian Haas (Nairobi), Andreas Kunkel (Berlin), Marie-France Leccia (Nice), Benoît Lequette (Réunion), Bart Meganck (Tervuren), Patricia Mergen (Tervuren), Dominique Nandancé (Tervuren), Martin Nebel (Stuttgart), Monika Pfeffer (Stuttgart), Marian Ramos (Madrid), Patrizia Rossi (Valdieri), David Schindel (Washington, D.C.), Eduard Stloukal (Bratislava), and David Tarkhnishvili (Tbilisi) for their generous help and kind support granted in many different but essential ways for the project. Also, we would like to thank the very many colleagues, here unnamed, who contributed significantly to the completion of this volume by reviewing and commenting on individual parts and manuscripts.

A special word of thanks goes to Dr P. Bridgewater, Chairman of UK and international nature conservation advisory organisation JNCC (Joint Nature Conservation Committee), for providing the text on the back-cover.

The permanent (Y. Samyn, D. VandenSpiegel and J. Degreef) and *ad hoc* associated (J. Eymann, Ch. Häuser and C. Monje) editorial board of this volume of *Abc Taxa* would like to express its gratitude to all who have generously aided in the realisation of this book.

Funding for the production of this book came from the Belgian Directorate General for Development Cooperation through the Belgian National Focal Point to the Global Taxonomy Initiative and from the Sixth Framework Programme (FP6), Sub-Priority 1.1.6.3 "Global Change and Ecosystems" (project no. 018340), of the European Union through the European Distributed Institute of Taxonomy, partim WP7.

About the authors

Marina Aboal (º1959) is Full Professor and the head of the Phycology Research Group at Murcia University (Spain). She is interested in biodiversity and ecology of freshwater algae from the Mediterranean area. Although having studied lenitic and flowing systems in different regions she addressed in last years the algae communities and functioning of semiarid streams and the production of cyanotoxins by benthic communities. Her current research focuses on systematics of filamentous algae and its relationship with phosphorus dynamics.

Miguel Ángel Alonso-Zarazaga (°1956) is Tenured Scientist (Entomologist) at the Spanish National Museum of Natural History in Madrid (MNCN-CSIC). He carries out research on the nomenclature, systematics and evolution of Coleoptera Curculionoidea (weevils), particularly of the families Apionidae and Nanophyidae, and on the fauna of the Western Mediterranean and Macaronesia. He has described several new taxa of hypogean weevils and has more in study. Currently he has more than 130 published papers and, in collaboration with C.H.C. Lyal, runs a weevil database, WTaxa (http://wtaxa.csic.es).

Yves Basset (°1960) is scientific coordinator of the Tropical Canopy Biology Programme and Arthropod Initiative of the Center for Tropical Forest Science at the Smithsonian Tropical Research Institute in Panama. His main research interests are plant-insect interactions and canopy arthropods in tropical rainforests. During the past 25 years his professional residence was in Australia, Hawaii, Papua New Guinea, Guyana and Panama, with other field projects completed or extent in Gabon, Brasil and Thailand. Currently he is leading an ambitious programme to monitor arthropods in tropical forests.

Philippe Bouchet (°1953) is senior professor at the French National Muséum of Natural History, in Paris. He is a malacologist whose interests are with the exploration and description of biodiversity, especially marine invertebrates. Exploring for unknown faunas has taken him to expeditions in three oceans, as well as to a number of remote islands, with emphasis on the western Pacific, where he has coordinated several major international expeditions that have and have rallied the forces of as many as 80 scientists from 19 different countries working together to change our perspectives on marine megabiodiversity, attracting attention of the public, policy makers and scientists alike.

Peter Bridgewater (°1945) is currently Chair of the UK Joint Nature Conservation Committee and has been deeply involved in international biodiversity discussions for over 20 years. He helped organise the 'Darwin Conference' in 1998 which spurred the acceptance of the Global Taxonomy Initiative (GTI) by the Convention on Biological Diversity (CBD).

Bart Buyck (°1959) is associate professor and curator of the national mycological herbarium at the Muséum national d'histoire naturelle in Paris, France. He is also president of the French mycological society. His main interests are phylogeny and taxonomy of ectomycorrhizal basidiomycetes, particularly Russulales and Cantharellales. He has an extensive experience with fungal inventories from the tropics to the arctic zone. He participated in the Great Smoky Mts national park ATBI (USA), the ATBI in Santo (Vanuatu) organized by the Muséum, and he presently organizes for EDIT the fungal inventory for the ATBI in the Mercantour (France) - Alpi Marittime (Italy) national parks.

Santos Cirujano (°1950) is a researcher at the Real Jardín Botánico (CSIC) in Madrid. He is a specialist in Aquatic vascular plants.

Jan Decher (°1960) is a mammalogist by training and a research associate and lecturer in the Biology Department of the Unversity of Vermont. His main research interests are the zoogeography, ecology, and conservation of West African small mammals. He has also worked as a small mammal consultant for the rapid assessment program (RAP) of Conservation international, for the environmental engineering firms NKUK and SNC Lavalin, in Côte d'Ivoire, Ghana, Guinea, and Sierra Leone, and recently for Heindel & Noyes, Inc. for an Environmental Impact Statement in southern Vermont.

Jérôme Degreef (°1966) is head of the department Cryptogamy and curator of the cryptogamic collections at the National Botanic Garden of Belgium, Meise. His research focuses on the taxonomy of tropical African fungi (mainly Boletales) and ethnomycology. During the past 10 years he has been involved in fieldwork all over Africa, collecting specimens for traditional morphology-based and molecular taxonomic research and compiling a checklist of edible mushrooms. He also is the co-founder and one of the permanent editors of the capacity building journal *Abc Taxa*.

Wouter Dekoninck (°1974) obtained his PhD in 2008 with a study on the distribution, ecology and conservation of ants in Belgium with emphasis on Flanders. Since 2000 he works at the Entomology Department of the Royal Belgian Institute for Natural Sciences in Brussels as scientific assistant, curator of databases and curator of the entomological collection. His main topics are sampling methodology, ecology and inventory of Formicidae, Carabidae and Culicidae; Red data analyses; Site Quality Assessment using invertebrates; knowledge on insect distribution, protection of threatened habitats/species. He is Belgian ant curator since 2006 and president of the Royal Belgian Entomological Society since 2009.

Jacques Delabie (°1957) is researcher at the Cocoa Research Center of CEPLAC, Brazilian Agriculture Ministry, and full Professor of Santa Cruz State University, Bahia, Brazil. His main research interests are ant biogeography, community ecology and diversity, as well as use of ants for biological control in cocoa plantations or biomonitoring. He has collaborated to several large research programs for ant taxonomy and ecology. He is currently participating to arborous and leaf-litter ant community and ant-plant interaction studies, firstly, but not exclusively, in the Neotropical Region.

Teresa Domingo-Quero (°1976) is a predoctoral fellow in the Spanish National Museum of Natural History in Madrid (MNCN-CSIC). She is currently finishing her PhD thesis on the Coleoptera diversity of the Caldera de Taburiente National Park (La Palma I., Canary Islands, Spain). In 1999 she graduated in Biology in the Alcalá University (Madrid, Spain). From 1999 to 2001, she worked in the ATBI project: 'The Invertebrate Fauna Inventory of Caldera de Taburiente National Park'. During two years of field work, she collected invertebrate samples using multiple techniques and processed them in the laboratory: soil, litter, vegetation, freshwater and caves were included in this study.

Gabriele Droege (°1978) is a computer scientist at the Botanic Garden and Botanical Museum Berlin-Dahlem. She studied Biology at the Freie Universität Berlin. After her Diploma thesis about rooks (*Corvus frugilegus)* she developed the Corvids Literature Database. Within the DFGfunded DNA Bank Network she is currently responsible for data architecture and software development. Her main interest is directed towards aggregation of biological data, especially all kinds of biological collections and publications.

Xavier Eekhout (°1977) is a herpetologist currently working in project management in the Museo Nacional de Ciencias Naturales (MNCN). Although he has mainly worked with amphibians, he also has strong personal interests in reptiles. Professionally he has studied frog population dynamics with the aid of drift fences, and later acoustic communication in anurans of Spain and Mexico. He has also participated as instructor in general bioacoustics courses in Mexico and regularly collaborates with the Fonoteca Zoologica (www.FonoZoo.com), the animal sound collection of the MNCN.

Jutta Eymann (°1972) has been working as scientific coordinator for the workpackage “Applying Taxonomy to Conservation” within the EU-project EDIT (European Distributed Institute of Taxonomy) while co-editing this Field Manual. Currently, she is back in Australia where she did her PhD on conservation and management of urban brushtail possum populations.

Jon Fjeldså (°1942) is curator of birds at the Zoological Museum, University of Copenhagen, since 1971, and is now also professor in biodiversity studies. Has long experience in expedition work in tropical countries, especially in the Andes of South America and East Africa.

Andreas Floren (°1961) is since 18 years involved in canopy research focussing on the diversity, structure and dynamics of tree specific arboreal arthropod communities, which are sampled mainly by highly efficient canopy fogging. Starting in SE-Asian primary rain forests he extended his research to anthropogenically disturbed forests. For about 12 years he is carrying out comparable studies in temperate Europe. There is no lack of managed forests in Germany, but for primary forests he had to change to the polish Bialowieza forest. These data form the basis for his recent research project focussing on the functional importance of xylobiontic arthropods (mainly beetles) by manipulating key resources like canopy and ground dead wood.

Antonio Garcia-Valdecasas (°1952) is a researcher at the Museo Nacional Ciencias Naturales (CSIC) in Madrid. He is a specialist in Hydrachnidia and has worked on taxonomic tools by computer. Presently he is working on the scientific foundations of taxonomic work.

Birgit Gemeinholzer (°1968) is scientific head of the molecular laboratory at the Berlin Botanic Garden and Botanical Museum (Freie Universität Berlin). She is heading the German DNA Bank Network and is involved in several projects to safeguard primary data of biodiversity research. Her own research interest is within the plant group Cichorieae/Asteraceae analyzing evolutionary and phylogenetic mechanisms but also covering topics on species delimitation and detection, like DNA barcoding.

Adriaan Gittenberger (°1976) is associate researcher at the National Museum of Natural History Naturalis, and the Institute of Biology, Leiden University (The Netherlands). He joins the marine expeditions of Naturalis as specialist of ascidians and gastropod parasites of corals. In addition, he directs GiMaRIS (cf. www.gimaris.com). Together with his team of biology and bioinformatics students he runs a multidisciplinary research program in temperate areas, focusing on marine species communities and population dynamics based on long-term monitoring, field studies and aquarium experiments and molecular analyses.

Robbert Gradstein (°1943) is a retired Professor of Botany, and former Director of the Herbarium and Botanical Garden of the University of Göttingen. His main research interests are systematics and biogeography of bryophytes, especially liverworts, and flora and vegetation of the tropics. Professor Gradstein had done fieldwork in all parts of tropical America and in Asia, has directed numerous research projects in his field of interest, and has published more than 350 research papers, including several books. He is co-author of "Guide to the Bryophytes of Tropical America" (2001), the first synthesis of its kind. Professor Gradstein is the editor-in-chief of the journal TAXON, member of the Academy of Sciences of Göttingen, and a former president of the International Association of Bryologists. For his botanical research he was awarded the Prix Augustin-Pyramus de Candolle of the Societé de Physique et d'Histoire Naturelle de Genève.

Patrick Grootaert (°1952) promoted with a PhD on freeliving nematodes at the University of Ghent, but later specialised in Diptera, Empidoidea when he became employed at the Royal Belgian Institute of Natural Sciences in Brussels in 1980. In the beginning he worked on taxonomy and faunistics of Palaearctic fauna. He helped to develop Red Data Books and performed many site quality assessments of forests, heath land and coastal dunes using various insect groups as indicators. He directs the Department of Entomology at RBINS since 1991 and his main interests are now in the fauna of Southeast Asia and in particular in mangrove fauna.

Michael Grundmann (°1961) is curator for molecular collections at the Botany department, Natural History Museum, London. His doctoral thesis, prepared at Heidelberg University, Germany and the NHM, was on phylogeny, population genetics and phylogeography of the moss genus *Pleurochaete*. During the past 10 years he has been involved in fieldwork all over Europe, collecting specimens for molecular research on bryophytes, ferns and flowering plants. His current work focuses on conservation and storage procedures of DNA and tissue collections.

Christoph L. Häuser (°1959) is a biologist with a background in taxonomy and evolutionary biology, originally specializing in the study of Lepidoptera. Since 2009 he is leading the office for international collaboration and science policy at the Directorate of the Berlin Museum für Naturkunde, and has been actively involved in several international efforts linking taxonomy, information science, and biodiversity conservation (CBD: GTI, GBIF). For the EDIT project, he has been leading Work Package 7 (Applying Taxonomy to Conservation), developing an ATBI+M approach for protected areas. He has ample field experience as an entomologist working recently at ATBI+M pilot sites in Europe, and also before in many countries in Asia, Africa, and Latin America.

Anke Hoffmann (°1962) is scientific coordinator for the workpackage “Applying Taxonomy to Conservation” within the EU-project EDIT (European Distributed Institute of Taxonomy) and is based at the Museum für Naturkunde in Berlin. Her PhD (1999) was on population dynamics and habitat utilisation of terrestrial small mammals in the grasslands of Queen Elizabeth National Park in Uganda. Moreover she has been involved in further small mammal surveys in different habitats, like in rain forests in Uganda and Guinea, also in the Nama-Karoo in Namibia. Currently she focuses on small mammal inventories in the EDIT ATBI+M sites of Slovakia (Gemer Area) and France/Italy (Mercantour/Alpi Marittime National Park).

Joachim Holstein (°1965) is research entomologist at the State Museum of Natural History Stuttgart, Germany. He is interested in afrotropical Saltatoria and Araneae, mainly Jumping Spiders (Salticidae) but is also very familiar with the insect fauna of Central Europe. His wide-ranging interest in entomological subjects as well as in IT technics results in several projects of designing database management systems for collection management, faunistics, and research. His current research focuses on East African jumping spiders and the insect fauna of Kakamega Forest in Western Kenya as well as on the development of an illustrated database and information system for the European fauna.

Valerie Hofstetter (°1964) is responsible for the molecular diagnostics of fungi at the Agroscope Changins-Wadenswil, Nyon, Switzerland. She is a molecular biologist interested in systematics of various groups of asco- and basidiomycetes. She participated in fungal inventories on many continents, where she had the responsibility for the barcoding aspects and tissue sampling for all fungal groups. She presently works on fungal communities of vine and other agricultural crops.

Sanda Iepure (°1974) is a Researcher at Speleological Institute “Emil Racovitza” Biospeleology Department from Cluj, Romania. She carries out systematic, ecologic, biogeographic and conservation researches on groundwater crustaceans (cyclopoids and ostracods) mainly from caves. Currently her researches are focused on speciation and phylogeny of cave cyclopoids and ostracods from the peri-Mediterranean region inferred from both molecular and morphologic approaches.

Damià Jaume (°1963) is CSIC staff Scientist at the Mediterranean Institute of Advanced Studies (Balearic Islands, Spain). His research is focused on the taxonomy, comparative morphology, biogeography and phylogeography of peracarid crustaceans, especially those living in anchialine environments and other subterranean habitats.

Lars Krogmann (°1976) is a research scientist and curator of Hymenoptera in the Entomological Department of the State Museum of Natural History in Stuttgart. His research focuses on the systematics and evolutionary biology of parasitoid wasps. He obtained a PhD from the University of Hamburg and has previously worked as a Feodor Lynen Fellow of the Alexander von Humboldt-foundation at the University of Adelaide in South Australia.

Alexander Kroupa (°1966) is graduated in Zoology focussing on community ecology and systematics at the University of Bayreuth and specialised on aculeate Hymenoptera (www.HymIS.eu). Subsequently working for several companies and consultancies developing IT applications for various biodiversity monitoring and conservation programs. Currently employed at Museum für Naturkunde in Berlin working for EDIT to test and improve field-based digital recording tools and techniques for biodiversity inventorying and monitoring programs.

Thomas Læssøe (°1958) is associate professor at the University of Copenhagen (Denmark) with an extensive field experience on many continents and in many climatic zones, from far Eastern Russia to lowland Ecuador. His main interests are with the taxonomy of ascomycetes, in particular pyrenomycetes (especially tropical Xylariaceae), but also has extensive experience with many saprotrophic basidiomycetes and several other fungal groups. He coauthors one of the most comprehensive, user friendly, multientry, computerised keys to fungi on and off the web (www.mycokey.com), maintains a webpage and database on Ecuadorean fungi, and co-runs the newly started atlas project in Denmark (www.svampeatlas.dk).

Gaël Lancelot (°1981) is the communication officer for the European Distributed Institute of Taxonomy, working at the Muséum National d'Histoire Naturelle in Paris. He has previously worked for the Global Biodiversity Information Facility and the BioCASE project. He is the author of *University Reorganisations in Biology: remodelling British and French life sciences, 1960-1995.*

Maurice Leponce (°1964) is researcher at the Biological Evaluation section of the Royal Belgian Institute of Natural Science. His main research interests are ant and termite community ecology and biodiversity. He has collaborated to large-scale biodiversity inventories in Panama, Australia, Vanuatu and France in the framework of IBISCA (Investigating the Biodiversity of Soil and Canopy Arthropods) projects. He currently studies the response of ant communities to environmental changes in Podocarpus National Park (EDIT's ATBI site in Ecuador).

Diego Llusia (°1978) is a PhD researcher in the National Museum of Natural Sciences (MNCN-CSIC) at Madrid (Spain). His research includes acoustic communication, behaviour and ecology of terrestrial vertebrates. He is currently involved in acoustic monitoring of anuran populations in light of climate change. He previously taught biology in SEK-Segovia University and was member of the Ecology of Steppe Birds Research Group in the Autonomous University of Madrid.

Raphael Marquez (°1962) is Tenured Researcher (Investigador Científico) of the Spanish National Research Council (CSIC). He directs the laboratory of Bioacoustics and the Animal Sound Library Collection of the National Museum of Natural Sciences in Madrid, Spain. His current research involves the development of acoustic monitoring programs in terrestrial habitats (anurans and birds).

Christopher Meyer (°1966) is a research zoologist at the Smithsonian National Museum of Natural History in Washington D.C. His main research interests are in diversification processes and patterns in marine systems, especially those associated with coral reefs. Generally, he uses diverse molluscan groups to test phylogenetic and phylogeographic hypotheses. His other research interests include the use of genetic markers to examine diversity in marine communities. Currently he is the director of the Moorea Biocode Project, an all taxon, voucher-based, genetic inventory of a tropical ecosystem in French Polynesia.

Marianne Meyer (°1943) is living in the French Alps. Notwithstanding the fact that she is an 'amateur' mycologist, she is one of the world experts on myxomycetes maintaining an intensive international collaboration and activity. She is particularly known for her contributions on nivicolous myxomycetes and is presently finishing a prestigious and beautifully illustrated world monograph. Her incredible energy and contagious enthusiasm for myxomycetes has made her one of Europe's best known ambassadors of this group.

Juan Carlos Monje (°1963) is a scientist at the State Museum for Natural History, Stuttgart, Germany. His expertise is in the classical and molecular taxonomy of minute parasitic wasps. He works at present as project coordinator and is one of the team leaders for EDIT's Work Package 7.

Alexandra N. Muellner (°1974) is a postdoctoral lab manager and research group leader at the Grunelius-Moellgaard Laboratory at the Senckenberg Research Institute in Frankfurt, and teaches at Goethe University. She set the stage for Senckenberg´s DNA Bank and DNA database which cover various groups of organisms (plants, fungi, animals). Her research focus during the past 10 years has been on molecular phylogenetics, molecular phylogeography and historical biogeography of temperate and tropical flowering plants. Recently, research has centered largely upon taxa of the pantropical family Meliaceae (mahogany family) in the order Sapindales. Her scientific research resulted in various papers, book chapters (incl. floras), reports and media involvement. She has received several prizes and awards for her work.

Dirk Neumann (°1972) is diplom biologist and technician managing the ichthyological collection and in charge for the technical equipment in the DNA Lab of ZSM. Main interests are the ichthyofaunal diversity of Bavarian rivers and nilo-sudanian rivers in Northwest Africa. Besides coordination in the species inventory of Bavarian freshwater fishes, he was engaged in 8 field projects in South America and Africa the past 10 years.

Martin Obrist (°1958) is research associate in the Biodiversity and Conservation Biology research unit of the Swiss Federal Research Institute WSL in Birmensdorf, Switzerland. He is engaged in the development of biodiversity assessment tools in general as well as acoustic tools for the assessment of bat species by their echolocation calls in specific. Present work involves the acoustics part of the revision of the Red List of Swiss bats and a study on bats' response to habitat connectivity, which both will be analysed in the framework of spatial ecology.

Beáta Papp (°1965) is the curator of the Bryophyte Herbarium (BP) of the Hungarian Natural History Museum since 1999 she is also the editor of *Studia Botanica Hungarica.* She participated in numerous monitoring and conservation programs, in her home country Hungary but also in the Balkan, Turkey and in East Asia. In 2005 she received the Pro Natura Award from the Ministry of Environment for her efforts in the field of conservation of bryophytes.

Gustav Paulay (°1957) is curator of marine invertebrates at the Florida Museum of Natural History. He uses integrative taxonomy, large-scale biodiversity surveys and sequencing efforts to document and understand the diversity, distribution, and evolution of reef-associated marine invertebrates. His focus has been largely on tropical Indo-Pacific corals, molluscs, crustaceans, and echinoderms.

Gianni Pavan (°1960) is Professor of "Terrestrial and Marine Bioacoustics" at the University of Pavia, Italy. He is President of the "Centro Interdisciplinare di Bioacustica e Ricerche Ambientali" he contributed to create in 1989 to develop advanced bioacoustic research based on digital techniques. He started to work on computational bioacoustics in 1980. His main research interests are now on marine mammals acoustics and terrestrial landscapes. Cooperates with ONR, NATO, WHOI, IT Navy and other institutions worldwide to study marine mammals. He developed the SeaPro package for real-time sound analysis and display.

Gitte Petersen (°1963) is professor in molecular systematics of higher plants at the Natural History Museum of Denmark. Her research is focused on phylogeny and molecular evolution of the monocotyledons.

Mark Pollet (°1961) was trained as an entomologist at the University of Ghent (1983) where he also accomplished his doctoral research on the feeding ecology of carabid beetles (Coleoptera, Carabidae) in pasture ecosystems. Since 1985 he grew a passion for scientific research on long-legged flies (Diptera, Dolichopodidae) from the Palaearctic and the New World, in all their aspects. As grant coordinator – scientific advisor sr at the Institute for the Promotion of Innovation through Science and Technology (IWT-Vlaanderen), between 1994 and 2009 he organized hundreds of evaluation committees and evaluated a multifold of PhD applicants in biology. Since 2009, he's head of the new Information and Data Center at the Research Institute for Nature and Forest (INBO), which combines his interest for nature conservation and information and data systems.

Heather Proctor (°1964) is a professor in Biological Sciences at the University of Alberta, Canada. Her early research was focused on the ecology and evolution of water mites (Hydrachnidia) but has since branched out to include the community ecology of freshwater and terrestrial invertebrates and the ecology and systematics of bird-associated mites.

David Rawson (°1943) is professor of Applied Cell Biology in the LIRANS Institute of Research in the Applied Natural Sciences at the University of Bedfordshire (United Kingdom) and Visiting Professor at the China Agricultural University, Beijing (China). His main area of research is cryobiology and the cryopreservation of cells, gametes and embryos of fish, and the study of cryo-impact on gene expression and sub-cellular structures. His is currently leading the development of cryo-banks of cells and tissues of UK fish species and also co-ordinating the collection and banking of material from critically endangered fish species worldwide as part of the Frozen Ark Project.

David Remsen (°1962) is a senior programme officer for the Global Biodiversity Information Facility in Copenhagen, Denmark. He works on the development of taxonomic infrastructure as a component of the GBIF network. His focus is on the development of taxonomic name services that include authoritative catalogues of common and scientific names of species and the application of these names, and information about their relationships, to the access and retrieval of information about species. He is the architect of the uBio Project in the USA and is a member of the EDIT Strategic Advisory Council.

Swen Renner (°1974) is Research Scientist at the Institute of Experimental Ecology at the University of Ulm. His research is mainly about biogeography and taxonomy of SE Asian birds and the impact of land use on bird communities. His work involves a wide variety of methods including museum collection work, field work as well as GIS and lab analysis.

Isabel Rey (°1961) is the curator of Tissues and DNA of the Museo Nacional de Ciencias Naturales of Madrid. Her research is focused on preservation of DNA and on methodology to obtain ancient DNA from classical zoological collection specimens. In addition, she is involved in designing standard protocols to obtain non invasive samples of living animals.

Klaus Riede (°1953) is Associated Researcher at the Zoological Research Museum Alexander Koenig, Bonn (Germany). His research focuses on neuroethology, bioacoustics and ecology of Orthoptera, with a strong focus on tropical species. He managed major biodiversity database projects, such as a global geo-database on migratory species (Global Register of Migratory Species) and a web-based multimedia database on Orthoptera specimens with song recordings. At present, he is working on cricket sensory ecology at the Smithsonian Tropical Research Station at Barro Colorado Island (Panama), with funds from the FWF Austrian Science Fund to the University of Graz.

Francesco Rovero (°1970) is the Curator of the Tropical Biodiversity Section at Italy's Trento Museum of Natural Sciences. Since 2002, he conducts research on diversity, ecology and conservation of rainforest mammals in the Eastern Arc Mountains of Tanzania, with a focus on census methods and ecological modeling of primates and ungulates. He works predominantly in the Udzungwa Mountains, where he helped establishing, and currently directs, the Udzungwa Ecological Monitoring Centre, a field station annexed to the Udzungwa Mountains National Park. He holds a PhD in Animal Ecology at the University of Wales in Bangor (UK) and published over 40 scientific papers and book chapters.

Yves Samyn (°1972) is employed by the Royal Belgian Institute of Natural Sciences in Brussels (Belgium) where he is coordinator of the activities of the Belgian National Focal Point to the Global Taxonomy Initiative. In this capacity he organises training for students and young researchers from developing countries in particular from Africa. To this end he has developed a web site focused on capacity building in taxonomy (www.taxonomy.be). He also is the co-founder and editor-in-chief of the capacity building journal *AbcTaxa*. His research expertise is on the taxonomy of sea cucumbers (www.echinodermata.be).

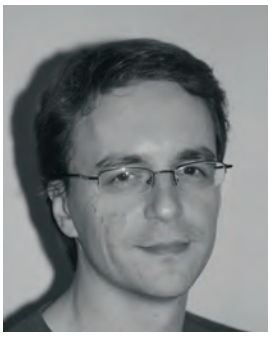
Jim Sanderson (°1949) is the founder of the Small Wild Cat Conservation Foundation, a Fellow of the Wildlife Conservation Network, a conservation officer of the Feline Conservation Federation, and one of the world's leading authorities on small wild cats. He has studied the Guigna, assisted his colleagues in the capture of the Andean cat, Margay, and Jaguarundi. With his Chinese and Tibetan colleagues, he obtained the first pictures of the Chinese mountain cat taken in the wild. He has a Ph.D. in mathematics and also wildlife ecology and conservation. He is the co-author of four books, and published more than 100 peer-reviewed articles.

Juliane Schaer (°1982) is a MSc student of biology at the Humboldt University in Berlin. Her main research interests are systematics, evolution and ecology of Chiroptera. During the past 4 years she has worked in the mammal collection of the Museum für Naturkunde in Berlin and also has been involved in field trips to Costa Rica and two small mammal surveys in Guinea. Currently, she is investigating host associations and evolutionary relationships of West African chiropteran blood parasites as part of her diploma thesis at the American Museum of Natural History, New York.

Ole Seberg (°1952) is Professor at the Laboratory of Molecular Systematics, Natural History Museum of Denmark, Copenhagen. His main research is in Molecular Systematics and Molecular Evolution especially on the phylogeny of the Monocotyledons and the evolution of the plant mitochondrial genome.

Mark R. Shaw (°1948) is the former Keeper of Natural Sciences and is now honorary research associate at the National Museums of Scotland, Edinburgh. He has published about 200 papers on parasitic wasps, particularly on their host associations, ecology and developmental biology, but also including some faunistic, taxonomic and phylogenetic contributions. The main focus has been on Ichneumonoidea, especially those parasitizing Lepidoptera and spiders, and to a much lesser extent Chalcidoidea.

Axel Steiner (°1960) is a free-lancing biologist with a background in archaeology who has worked on the Lepidoptera fauna of Germany at the Staatliches Museum für Naturkunde in Karlsruhe. He has a long-standing experience in collecting nocturnal Lepidoptera and in monitoring species for conservation projects. At the Staatliches Museum für Naturkunde in Stuttgart he was involved in the development and maintenance of taxonomic databases of butterflies. His interests include insect photography, the history of entomology and of entomological illustration, and he has done some taxonomical work on Noctuidae (Lep.)



Jerome Sueur (°1972) is Assistant Professor at the Muséum national d'Histoire naturelle, Paris. He is interested in systematics and biomechanics. One part of his research focuses on the acoustic behaviour of insects with a particular attention to mechanical auditory processes of cicadas. He is developing a new method to assess and compare local biodiversity through acoustic analysis. He is the maintainer of seewave, an R package dedicated to sound analysis and synthesis.

José Templado (º1957) is a Researcher of the Spanish Research Council (CSIC) at the National Museum of Natural History of Madrid. He is mainly interested in reproductive biology, systematics and evolutionary diversification of marine gastropods. He has been involved in many fieldworks and oceanographic cruises. His scientific research resulted in over 110 scientific publications (including papers in scientific journals, books and chapters of books).

Conny Thiel-Egenter (°1976) is co-owner of FORNAT, a consulting company for nature conservation in Zurich, Switzerland. She is involved in projects on plant conservation, vegetation ecology and protected area management. During her PhD in the EU-project IntraBioDiv, she gained broad experience in floristics, plant biogeography and phylogeography. She has special interest in arctic-alpine flora and ecosystems.

Simon Tillier (°1950) is senior Professor at the Muséum national d'Histoire naturelle in Paris, and the leader of EDIT. He studied taxonomy and phylogeny of Molluscs and was the curator of land and freshwater molluscs at the Muséum national d'Histoire naturelle from 1976 to 1990. During this period he collected invertebrates in various regions of the world, particularly in New Caledonia, spending in total several years in fieldwork. From 1990 Simon Tillier turned to molecular systematics and phylogeny, and set up and directed the molecular systematics facility of the Museum. In parallel he got involved in organization of research in systematics at International, National and Museum levels.

Mathias Tobler (°1975) is a research ecologist in the Botanical Research Institute of Texas. He works with the Andes to Amazon Biodiversity Program on research and conservation in the Peruvian Amazon, with a focus on the ecology of Amazonian ungulates. He has a strong interest in developing new monitoring techniques and quantitative methods for data analysis. He is also involved in the development of new computer software tools for managing and analyzing large amounts of biodiversity data. He holds a Ph.D. in Wildlife Sciences from Texas A&M University.

Kees Van Achterberg (°1948) he made forty years ago his first Townes type Malaise traps to collect Braconidae (Hymenoptera). Both in Europe and SE Asia he experimented with modified designs of the traps and methods to prepare specimens from 70% alcohol. During the 2007 RMNH-IEBR expedition to S Vietnam he used 35 Malaise and Schacht traps simultaneously. He published 300+ papers and books on the taxonomy, phylogeny, biology and biogeography of Hymenoptera (Braconidae, Heloridae, Stephanidae, Ichneumonidae (Hybrizontinae), Pamphiliidae, Cephidae, Argidae and Thynnidae) and works currently as senior researcher at the National Museum of Natural History (Naturalis).

Didier VandenSpiegel (°1961) is employed by the Royal Museum for Central Africa, Tervuren (Belgium) where he is senior researcher in the Department of Zoology, section Invertebrates, non-insects. He also is the co-founder and one of the permanent editors of the capacity building journal *Abc Taxa*. He has research expertise in the taxonomy of echinoderms and millipedes.

Alain Vanderpoorten (°1974) graduated as a bioengineer and did his PhD at the faculty of agricultural sciences of Gembloux (Belgium). After several post-doctoral research programs on the phylogeny and evolution of aquatic bryophytes at Duke University (USA) and diversification of Proteaceae in the fynbos of South Africa at Rhodes University (RSA), he is now a research associate of the Belgian Funds for Scientific research. His research presently focuses on the evolution of island endemism in bryophytes.

Jackie Van Goethem (°1943) is Honorary Head of the Department of Invertebrates at the Royal Belgian Institute of Natural Sciences in Brussels. He was responsible for the curation of a worldwide-recognised reference collection of molluscs and other invertebrates. He also was Belgium's National Focal Point to the Convention on Biological Diversity and the promoter of a major project funded by the Belgian Development Cooperation for capacity building in taxonomy and collection management for developing countries. He is the Executive Secretary of the King Leopold III Fund for Nature Exploration and Conservation which offers opportunities for biodiversity related fieldwork outside Europe. His research interests cover a.o. terrestrial snails and slugs from Europe, Africa and New Guinea.

Jose Luis Velasco (°1947) is a researcher at the Museo Nacional Ciencias Naturales (CSIC) in Madrid. He is a specialist in Rotifera.

Christian C. Voigt (°1966) is a Senior Research Scientist at the Leibniz Institute for Zoo and Wildlife Research and also a lecturer at the Humboldt University in Berlin (Germany). His main research interests are the behavior, ecology and physiology of mammals, in particular bats. He combines novel methodological approaches such as stable isotopes with conventional techniques such as behavioral observations or ecological surveys. His research focus has been in the New World with a strong focus on Central and South American bat assemblages, but recent projects have brought him in contact with the African continent as well.

Lee Weigt (°1960) is the Head of the Smithsonian Institution's Laboratories of Analytical Biology (L.A.B.) at the National Museum of Natural History. Since 1985 he has managed and directed molecular laboratories at several major museums, research institutes and universities. His current efforts are focusing on bringing together cutting edge biotechnology and bioinformatics tools to the Smithsonian scientists studying biodiversity, and on sharing information amongst research efforts. His personal research interest surrounds recently diverged species and species complexes.

Kurt Weising (°1954) is full professor at the University of Kassel. His research is focused on the phylogenetic reconstruction and evolution of plants. Research projects cover intra specific as well as higher taxonomic ranks and use traditional taxonomic (anatomy, morphology, etc.) as well as DNA-based molecular methods (*e.g.* micro satellites, DNA sequencing).

Gudrun Wibbelt (°1971) is senior veterinary pathologist at the Leibniz Institute for Zoo and Wildlife Research, Berlin (Germany). Her research focuses on pathology and infectious diseases of wildlife animals. She is particularly interested in diseases in bat species and is working on this subject since some years.

Thomas Wilhalm (°1965) is curator of Botany at the Museum of Nature South Tyrol in Bolzano/Bozen (Italy). He is interested in floristics and taxonomy (especially of alpine species and of Poaceae) and has acquired a broad experience in these topics during the project "Mapping the flora of South Tyrol" which he manages. Other activities include setting up herbarium BOZ, of a computerized database of floristic records and updating the Red List data of endangered vascular plants of South Tyrol. He is also involved in national and international projects concerning floristic mapping, molecular biogeography and taxonomy.

Friederike Woog (°1968) is curator of the bird collection at the State Museum for Natural History in Stuttgart, Germany. In 2003, she started a long-term study on site fidelity and biology of rainforest birds in Madagascar.

Holger Zetzsche (°1971) is a postdoctoral botanist at the Botanic Garden Botanical Museum Berlin-Dahlem and Germany. During the last 3 years he has been involved in the establishment of the DNA Bank Network. He is interested in long-term DNA storage, DNA based plant identification as well as systematics and evolutionary diversification of *Pulsatilla*. Although trained as a classical botanist he acquired a broad knowledge of molecular tools on various taxa of plants and algae.

Elke Zippel (°1968) is a researcher at the Botanic Garden and Botanical Museum Berlin-Dahlem, Germany and project manager for the National Seed Bank for Crop Wild Relatives in Germany. Her further interests include population genetics and phylogeography of rare plants in Central Europe, ecology and dynamics of bryophyte vegetation, as well as the flora and vegetation of the Canary Islands.

Previous titles in this series

Taxonomie des holothuries des Comores
Y. Samyn, D. VandenSpiegel and Massin C.
Abc Taxa Vol 1 - 2006

Détérioration des collections de coquilles
R. De Prins and E. Rour (traduction)
Abc Taxa Vol 2 - 2007

Taxonomy of the *Cryptocarya* species of Brazil
P.L.R. De Moraes.
Abc Taxa Vol 3 - 2007

Guia taxonomica de los anfibios de Cuba (with Audio CD)
L.M. Diaz and A. Cadiz
Abc Taxa Vol 4 - 2008

Introduction to the taxonomy of the amphibians of Kaieteur National Park, Guyana
P.J.R. Kok and M. Kalamandeen
Abc Taxa Vol 5 - 2008

Sri Lankan Seaweeds – Methodologies and field guide to the dominant species
E. Coppejans, F. Leliaert, O. Dargent, R. Gunasekara and O. De Clerck
Abc Taxa Vol 6 - 2009

The Bee Genera and Subgenera of sub-Saharan Africa
C. Eardley, M. Kuhlmann and A. Pauly
Abc Taxa Vol 7 - 2010

Manual on field recording techniques and protocols for All Taxa Biodiversity Inventories and Monitoring

If the Biosphere were a kitchen, this would be the cookbook every kitchen hand working in it would want to have. It is encyclopaedic in scope, without missing on detail where it's necessary.

The aim is to provide a guide to undertaking All Taxa Biodiversity Inventory+ Monitoring (ATBI+M), with the laudable aim of accelerating the integration of taxonomic knowledge with biodiversity conservation and management. The ATBI+M approach has its critics, but there can be little doubt that the more information we can garner at the species level of biodiversity, the better we will be able to mange it as a whole. Warning against the complacency of one-time surveys there is advice on groups from whales to waterbears; slime moulds to forest trees, and everything in between. Particularly pleasing is the inclusion of a broad range of habitats, especially marine and freshwater. Some chapters are also on unexpected but important survey methods including bio-acoustics, camera trapping, and on specimen handling and preservation. Each chapter also has plenty of "how to's" for preservation and heaps of leads for identification – even in one case the advice on what sort of accommodation actually works in the field! And, as pioneered in Central America, reference to how para- and amateur taxonomists (increasingly called citizen science) must be part of the process if the exercise is to work.

Even if you are not doing an ATBI+M you will find this book useful, even fascinating. There is much much more in it than "just" collecting and survey advice. It may not seem bedtime reading, but just try it is my advice. Happy inventorying!.

April 2010

Dr. Peter Bridgewater
Chair of the UK Joint Nature Conservation Committee
United Kingdom

WITH THE SUPPORT OF
THE BELGIAN
DEVELOPMENT COOPERATION .be

Abc Taxa